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Symposium on Absorption Mechanisms and the Malabsorption Syndrome

Transport of Amino Acids Into and Across Cells

HALVOR N. CHRISTENSEN, PH.D.* AND DALE L. OXENDER, PH.D.†

THIS symposium is concerned with the massive disappearance of nutrients from the alimentary canal and their reappearance in various body channels; more particularly with the intervening events and their disorders. What is the nature of these events? We are dealing with the problem of transport, usually of structurally-specific mediated transport, and often active transport.

An axiom of biochemical advance is that a phenomenon should be studied in simple form, if possible in isolated form, in order to understand it. Do the chemical reactions of transport across the intestinal mucosa exist in simpler form? We will try to show that the simpler, probably universal phenomenon of active transport *into* cells is fully capable of producing active transport *across* cells.

From the Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan.

*Professor and Chairman; † Research Associate, Department of Biological Chemistry.

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Figure 1 shows how this may occur: if a cell is able to concentrate a solute from the cellular environment, it should be able to release the solute at an elevated concentration into another separated segment of its environment. All that is required is that concentration be interrupted or inhibited with regard to the restricted segment (side B); or instead concentration might be stimulated or facilitated from the other part of the environment (side A). Undoubtedly cells of the intestinal mucosal do pump the amino acids into their interior as other cells do; indeed Hird[1] has shown that this is the case. The problem then is to show whether they have supplemented facilities for concentrating amino acids from their mucosal side, or handicapped concentrating ability on their serosal aspect; or whether instead they have an entirely unrelated apparatus for transcellular transfer, which seems unlikely to us. If their ordinary transport apparatus is asymmetric, we should like to know whether the polarization is humoral or anatomic in origin.

TRANSPORT ACROSS CELLS ARRANGED TO FORM A BARRIER

We recently demonstrated experimentally that concentrative uptake into cells can indeed

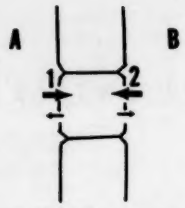

FIG. 1. Concentration into a cell can produce concentration across the cell. Inhibition of process 2 or stimulation of process 1 can produce accumulation in compartment B.

produce concentration across cells.[2] We have been able to form Ehrlich ascites tumor cells into an exceedingly thin membrane by catching them on a very fine cellulosic Millipore* filter. The filter very quickly becomes plugged to further filtration, a finding that had originally plagued us before we realized the usefulness of the resulting membrane. Cells that are not firmly attached are washed off with a saline solution. Figure 2 illustrates the microscopic appearance of these membranes in fixed sections prepared for us by Dr. Burton L. Baker (Department of Anatomy). Gravimetric determinations are in approximate agreement as to the illustrated number of cells forming the barrier.

When we placed a suitable saline medium (Krebs-Ringer bicarbonate solution) above and below this membrane, with 10 mM glycine in each phase, the only change in distribution was the uptake of glycine by the 5 or 6 mg. of cells present, causing an indetectible change in the glycine level of the two phases. We then tried to stimulate concentration at one face of the cells by placing 0.8 mM pyridoxal in the solution on that side. The membranes are sufficiently impermeable so that the pyridoxal gradient is not dissipated for several hours. After a twenty-minute lag the glycine level of the opposite phase began to rise, reaching a maximum level in about one hour averaging 1.17 times the concentration of the pyridoxal-containing phase (Fig. 3). The two opposed gradients then gradually disappeared together.

The phase volumes for this experiment were about 35 ml. for that containing pyridoxal,

* One inch type HA filters. Millipore Filter Corporation, Bedford, Massachusetts.

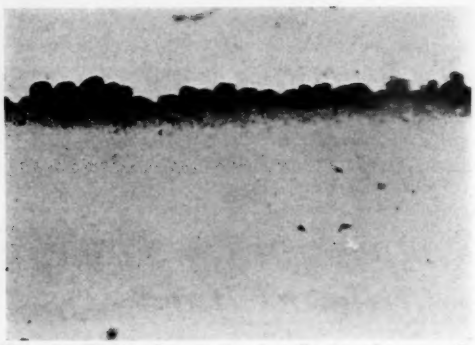

FIG. 2. Photomicrograph of cells in place on the Millipore filter, fixed with Bouin's fixative and stained with Regaud's iron hematoxylin and Masson's stain.

0.5 ml. for the other, the latter being kept small so that we would not have to wait too long for the concentrating ability to become apparent. Note how unlikely it is that these cells can release enough of a binding agent to bind 2 mM glycine in a 0.5 ml. volume, roughly 100 times the volume of the cells, or, considering both compartments, about 6,000 times that of the cells. Thus we have glycine being concentrated into a second extracellular compartment, where the glycine activity is very accessible to study, rather than into a mysterious cell interior where doubts can always be raised as to the free state of the amino acid.

This concentrating effect is independent of the relative position of the filter and the cells; in fact both surfaces of the cells may be covered with the filter material without significantly changing the result.

Pyridoxal itself is a binding agent for amino acids, but the stability constant for the resulting Schiff base is too low to make this a significant factor in the extracellular phases. In fact, if this factor operates at all, it would only intensify the problem of explaining the asymmetry of distribution.

Similar results are obtained with other α-amino acids including α-aminoisobutyric acid but not with N-dimethylglycine or α-methylglutamic acid, in precise agreement with the specificity of the intracellular concentration process. The process is similarly temperature-sensitive and dependent upon the presence of extracellular potassium (the latter acting to maintain an adequate level of cellular K^+).[3]

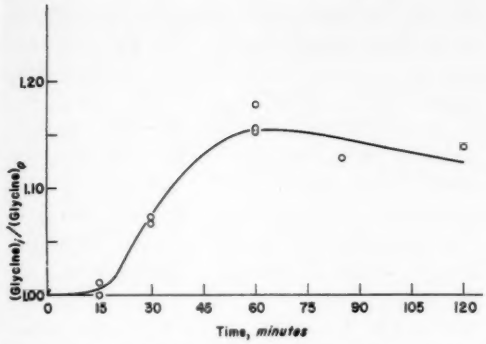

FIG. 3. Change of glycine distribution ratio with time after the addition of 8.0×10^{-4} M pyridoxal to phase o.

So far we have not been able to discover a single factor to which only one of the two processes is sensitive. Therefore we are convinced that intracellular concentration is serving to produce transcellular concentration. At the same time we believe this experiment has finally made unequivocal the evidence that transport into cell of these solutes is really active or concentrative. This conclusion, however, should not be freely extended to other solutes that are accumulated by cells.

Before we consider why pyridoxal produces this strange effect we should like to mention other means that we have used to produce asymmetry of amino acid distribution. If we set up a potassium gradient of 4 or 5 mM by initially placing 15 mM K^+ on the one (less voluminous) phase and omitting K^+ from the other phase (adjusting the Na^+ level in each case to maintain isoosmotic conditions) glycine moves for twenty-five minutes toward the solution (that is, losing K^+), to produce a distribution ratio of 1.1 (in one case 1.5) (Table I). This again recalls the behavior of these cells in giving up part of their potassium as they gain amino acid, and in being able to gain amino acid only in proportion to the K^+ they have available to lose.[3] Potassium ion penetrates the membrane more rapidly than pyridoxal, presumably mainly through spaces between the cells, since sucrose also gradually penetrates. This fairly rapid permeation of K^+ shortens the period during which this phenomenon may be observed and presumably limits its maximal intensity. Conversely, in analogy to concentrative uptake into the free cells, a potassium gradient arises across the membrane in the opposite sense to the glycine gradient when 50 mM glycine is placed on one side of the membrane (Table II). Accordingly, the idea gains strength that potassium migration may drive amino acid transport, and that the energy for both may be applied primarily to the maintenance of the potassium gradient,[3] the potassium gradient then serving to drive amino acid transport. This substantial evidence for common ground in the uptake of such highly dissimilar substances as amino acids and K^+ is interesting when we come to consider the specificity of absorption defects in diseases of intestinal absorption or of tubular transport.

As for the action of pyridoxal we note that

TABLE I

Effect of a Potassium Gradient on Glycine Distribution

Experiment	Final [Glycine] c.p.m./ml.		Ratio $[Glycine]_1/[Glycine]_2$
	Side 1	Side 2	
1	4,650	2,990	1.55
2	7,250	6,680	1.09
3	6,760	6,200	1.09
4	4,780	4,550	1.05

NOTE: Side 1 was made 15 millimolar in K^+ at zero time, whereas side 2 contained no potassium. At the time of observation (30 min.) the (K^+) was 4.5 mM on side 1, and about 0.2 mM on the voluminous side 2. The cells had been brought to equilibrium with 10 mM labeled glycine before preparing the membrane. The labeled glycine level was 10 mM initially on both sides of the membrane.

TABLE II

Effect of a Glycine Gradient on Potassium Distribution

Time (min.)	Final $[K^+]$mEq./L.		Ratio $[K^+]_1/[K^+]_2$
	Side 1	Side 2	
15	6.05	5.90	1.03
25	6.45	5.90	1.09
25	6.43	6.07	1.06
40	6.50	5.90	1.10
60	6.35	6.00	1.06

some moves onto the cells, staining them yellow, but this migration is almost instantaneous and not in step with the production of an amino acid gradient. The interior of the cell is apparently not generally accessible to the aldehyde. Prahbat Pal[4] has further shown in our laboratory that at least down to 0.5 mM pyridoxal levels the addition of neutral amino acids to the cell suspensions only decreases pyridoxal fixation by the cells despite the relative enrichment of the cell interior with a pyridoxal binding agent. Obviously the role for which we are considering pyridoxal here is as a possible carrier for amino acid transport. Although pyridoxal is chemically suitable for the types of carrier combination that must be considered,[5] the evidence, as recently summarized elsewhere,[6] is not entirely unequivocal for this role. At the levels through which vitamin B_6 varies in moderate depletion states, it is certainly a significant factor in the distribution of a model amino acid in the intact rat.[7] Dr. Masani Suda of the Institute for Protein Research, Osaka University, Japan, recently told us that depletion of vitamin B_6 by administration of penicillamine has permitted him to show a stimulating effect of pyridoxal on amino acid absorption from the intestine of the intact rat. Disturbances in placental transport of amino acids in toxemias of pregnancy[8] may be related to the apparently disturbed vitamin B_6 economy of pregnancy.

SOME ATTRIBUTES OF AMINO ACID TRANSPORT

We trust the foregoing results show that whatever properties may be found for the process of concentration of solutes into isolated cells are also significant for transmucosal transport. We may summarize some of the attributes that have been observed during a decade of preoccupation with this problem:[6,9,11]

(1) The amino acids fall into transport families. These divisions may depend on whether the amino acid contains a single carboxyl group or two, and whether it contains a single amino group or two. Within each of these families competitions characteristically occur, but not between members of different families. Some of the charged amino acids actually stimulate the transport of some of those of the neutral family. As Wiseman[10] has pointed out, the existence of such competitions may cause a partial stratification as to the levels in the upper intestine where various amino acids principally cross. The gradual release of amino acids by proteolysis may protect us to a considerable degree from excessive competitions for absorption. We also have to think about the competition among the amino acids for transport beyond the mucosa, into various cells, especially when amino acids are injected for nutritional purposes.

(2) For vigorous transport the amino group should be α or β to the carboxyl group, and if a second amino group is two or three but not more carbon atoms from the first, striking intensification of uptake occurs. These properties would make lower homologs of lysine and ornithine biologically dangerous, and may explain their limited occurrence. Effects on pyridoxal distribution completely different from those of other amino acids also result, suggesting pyridoxal binding within the cells by such amino acids.[4]

(3) Intensified transport may be signalled either by a decreased level at the site from which the amino acid derives or an increased level in the compartment into which it is pumped. Plasma amino acids, in relationship to tissue levels, frequently show the operation of this principle. Decreased plasma levels may signal that concentrative uptake has been stimulated (e.g., endocrinologically[11–13]) by a major tissue, or that an additional concentrative organ, for example the placenta in pregnancy, has been interposed.[13] The restraint of concentrative uptake may be essential for the transport of solutes from cell to cell, and may be a means for the control of growth.[11–13]

(4) Because active transport is subject to saturation, the concentration gradients at high loads may come to be in the reverse direction, and the anatomic barrier to diffusion which is necessary for effective concentration may become conspicuous.

(5) The energy sources for amino acid transport may be either oxidative or glycolytic and

do not appear distinctive, except for the possible channelling through the potassium pump to amino acid transport.

(6) The minimal reaction for transport (and or course we are interested in discovering the minimal reaction and not all the unessential fates that may follow) remains unidentified but involves primarily the amino group, and is sensitive to pyridoxal, a substance that reacts appropriately with the amino group.

SPECULATION

For this process we visualize the existence of a chemical reactant essential to transport. This reactant may become accessible to the transported solute despite the barrier to diffusion either by extruding a portion of itself to form an active site at the cell periphery or by receiving the solute secondarily from a superficial active site. The reactant could be visualized as able to receive the free solute from one aspect of a macromolecule forming part of the outer barrier of the cell, but able then to pass the solute on to the carrier only on the opposite aspect of the molecule[14] inside the osmotic barrier. In the latter case either reaction may serve to increase the energy level of the solute, so that it may be released into the interior of the cell at a higher concentration. If this process is less effective on one aspect of the cell, transcellular concentration will result. An osmotic pump that concentrates a solute outward (e.g., Na^+) may be similarly organized to produce transcellular concentration.

We might expect to isolate the transport reaction even further if we could shear off with an accurate microtome the one face of the cell barrier, to leave us with a single plasma membrane to study rather than two. We can perhaps achieve this purpose with a chemical "microtome" which can destroy the transport activity of the exposed surface. If we then place a suitable potassium-rich intracellular medium as one phase and an extracellular medium for the other, perhaps we can still obtain the characteristic transport activity. Note, however, that we lose the usual indicator of transport if we simply isolate cell membranes in a randomly orientated preparation. Such a preparation will be useful only if we discover a way to mark the transport reaction so we can recognize it in the absence of separate "extracellular" and "cellular" phases.

SUMMARY

The question of whether or not transport across the intestinal mucosa or similar barriers can be studied in simpler forms has been considered. We have shown that cells can be arranged to form a membrane able to concentrate amino acids from one and into the other of two phases separated by the membrane. This was achieved by adding pyridoxal or pyridoxal phosphate to the first phase, or by adding an excess of potassium ion to the second phase. The characteristics for the transport across the cells were exactly similar to those for accumulation into the cells.

These results show that the concentrative process for cells produces elevated levels of free amino acids, and is able to simulate the behavior of a secretory tissue. They further show that the attributes found for the transport into or out of cells are significant for transmucosal transport. Accordingly, these attributes have been summarized, and possible ways in which active transport may take place have been considered.

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DISCUSSION

QUESTION: Do enzyme inhibitors modify the ratio?

DR. CHRISTENSEN: The question was whether or not chemical inhibitors are able to modify the development of gradients across the artificial membrane of cells. This has been tried, and the answer is yes. Several inhibitors produce results in the membrane system rather similar to those produced in suspensions of the Ehrlich ascites tumor cells. However, the chemical agents used are often reactive with the pyridoxal with which the gradient is produced, for example cyanide or mercury or a number of other substances have been tried; so that the results are preliminary and not unequivocal.

We have felt that we ought to be able to produce an actual gradient with such agents inhibiting the transport on one of the exposed surfaces. But we need here to limit ourselves to inhibitors that influence carrier transport *per se* across the membrane, rather than those which interfere with the delivery of energy to the transport apparatus.

For example, we have thought of establishing anoxia on one surface of the cells by simply not having oxygen present in the corresponding atmosphere. But whether or not we could maintain an oxygen gradient across the barrier for an adequate length of time is rather dubious, in view of the diffusability of oxygen. Furthermore, oxygen deficiency may be expected to influence the availability of energy for transport at all surfaces of the cell, and not merely at the one exposed to anoxia.

Intestinal Absorption of Sugars and Amino Acids

J. H. QUASTEL, PH.D., D.SC.*

IT IS well known from the history of scientific progress that new results and new ideas come from the application of new technics. The study of the factors influencing intestinal absorption is now entering a fresh phase, and this is through the employment of novel methods. I will attempt to describe these methods and assess their relative advantages and disadvantages. None can be regarded as ideal but useful information may come from any one of them.

A method[1,12,15–17] used by older workers was the employment of ligated loops of intestine in the anaesthetised animal. One or more loops were separated from the mesentery leaving nerve and blood supply intact. The substance under investigation was placed in the ligated loop which was replaced in the abdomen. After a period of time the loop was emptied and the contents analysed. Disappearance of the substance under study was taken as an index of absorption. The drawbacks of the method are obvious. There are complications due to anaesthesia, to the difficulties of completely emptying the loop, to gastric motility and to metabolic events leading to changes of composition in the gut which are independent of intestinal absorption.

Cori[2,13] used fasted rats which were fed a known quantity of test substance by stomach tube. After a given time the animals were killed and the contents of the entire gastrointestinal tract were analysed (see also references 14 and 21). This method was later modified[3] to avoid force feeding but, although anaesthesia was eliminated, the other objections already mentioned were present.

The Vella fistula, with many modifications, has often been used.[4] The technic here is similar to that using the ligated loop except that one or both ends of the loop are brought to the surface of the abdomen. The advantage of the technic is that a number of experiments can be carried out with the same loop. The drawbacks are those inherent in the loop technic together with the objection that with repeated use of the same loop degenerative changes take place in the intestinal tissue which further obscure the results. Sols[5] used a modification of the fistula technic whereby the intestine *in situ* is canulated at both ends in an anaesthetised animal, but again the technic is complicated by the presence of surgical shock and trauma. The essential drawback to these *in situ* methods is that measurement of absorption is indirect, depending upon differences between initial and final concentrations of the test substance in the intestine and making no adequate allowance for metabolism independent of absorption.

Another approach has involved estimation of test substances in the blood or lymph of the animal.[6–9,18,20,22] While this technic gives information about the absorption of test substances, the quantitative aspects are limited, for the rates of absorption depend on such variables as blood flow and pressure, and the amounts of test substance present depend upon the manner in which other tissues in the body are involved in withdrawing the substance from the blood or lymph or in contributing to it.

In vitro technics were used at an early date.

From the McGill-Montreal General Hospital Research Institute, Montreal, Canada.

* Professor of Biochemistry, McGill University and Director, McGill-Montreal General Hospital Research Institute, Montreal.

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For instance, Auchinachie, MacLeod and Magee[10] studied glucose absorption by isolated intestine of the rabbit suspended in a beaker containing oxygenated Tyrode's solution. The lumen contained the test substance and the rate of its appearance was measured in the outer solution bathing the intestine. Selective absorption was observed although not consistently. Cordier and Touzem[11] also used intestinal loops taken from the rat. This method, although simple and capable of yielding useful information, suffers from the drawback that the mucosal solution in the lumen may not be adequately oxygenated. It is imperative for accurate work that the solutions bathing both aspects of the intestine be oxygenated.

New technics aimed at overcoming these drawbacks made their appearance during the last decade. Fisher and Parsons[23] devised a perfusion technic modified later by Wiseman,[24] for the study of absorption of substances across the wall of the isolated intestine of the rat, that is from the lumen of the intestine into the solution bathing its serosal aspect. The technic ensured proper oxygenation of mucosal and serosal solutions. It was difficult, however, with this apparatus to obtain samples at regular intervals, so that time-course studies could not be obtained with ease.

Independently of these investigators, Darlington and Quastel also devised a perfusion technic,[25,86] somewhat simpler in construction than that of Fisher and Parsons, which made it possible to study the kinetics of transfer of substances from the mucosal solution bathing the lumen of the intestine into the serosal solution bathing the serosal surface of the intestine under conditions approximating the physiologic. This technic, applied to the study of intestine of the guinea pig *in vitro*, has been used in a variety of investigations for the study of the factors influencing intestinal absorption. The advantages of the technic consist of its applicability to kinetic studies, of the ease by which substances may be added at any time to the mucosal or serosal solutions or by which samples may be withdrawn from either solution, of the manner in which constant circulation of fluids is effected and by which any gas, or mixture of gases, may be used in the study of intestinal metabolism and the relatively little damage (as tested by histologic examination) that is done to the intestinal preparation during the short time (thirty minutes to one hour) in which the apparatus is in use. Its disadvantages are that it is somewhat cumbersome (though it can be made with ease), that it cannot be used with ease to measure respiration of intestinal tissue nor to study the kinetics of transfer of water (unless H_2O^{18} or D_2O is used), that relatively large volumes of fluid are employed which make experiments with radioactive substances expensive and that only a small number of experiments can be carried out each day.

A simpler technic was devised later by Wilson and Wiseman.[26,27] This consists of preparing isolated small intestine of the rat or hamster in a manner that permits measurement of respiration and glycolysis during periods of transference of substances across the intestinal wall. The difficulty of adequate oxygenation of the mucosal surface is overcome by everting a piece of intestine, tying it at both ends and filling it with sufficient fluid to distend the wall. The eversion exposes the mucosa to the oxygenated medium while the distension increases the surface area of the sac and reduces the thickness of the wall of the sac. The oxygenation of the inner layer of serosal surface is facilitated by an oxygen bubble introduced into the sac along with the inner fluid. The advantage of this technic is that the relatively small volume of fluid contained in the sac (now the serosal side) allows a rapid rise of concentration of transferred substances. It is, therefore, well suited for studies of transport against a concentration gradient or for studies of transport of water. The technic, being simple, enables a relatively large number of experiments to be carried out each day. Its main disadvantages are that kinetic studies cannot be adequately carried out, a separate piece of intestine being needed for each point on a time curve; the oxygenation of the serosal solution (in the sac) is unknown and may vary from one experiment to the next or from one substrate to another; it is difficult to carry out experiments with different gases in the everted sac; the tissue is unavoidably

subject to considerable injury during the process of eversion, much more so than in the perfusion technic;[25] the amount of distension in the sac varies according to the experimenter (a possible reason for the disagreements among various groups of workers) and the peristaltic movements of a closed sac must affect movements of fluid in and out of the sac. In spite of these disadvantages, much can be learned from the everted sac technic. It seems that a combination of both this and the perfusion technic[25] is desirable for further progress in studies of intestinal absorption. Both technics have so far given results qualitatively in agreement with each other. It must be realized, however, that these technics are concerned with factors influencing transport of substances across the intestinal wall. Whether all results obtained in this way apply to the problems of absorption from the intestine into the blood still remain to be seen. So far, however, results obtained by *in vivo* or *in situ* experiments have been confirmed by the *in vitro* technics just described. It is likely that the results of *in vitro* work will apply *in vivo*, but there must be some reservation until experiments, to test the conclusions made from experiments made *in vitro*, have been carried out. A fact that must be borne in mind is that it is necessary to distinguish between various parts of the intestine in considering absorption processes.[84] Work is usually carried out with small intestine in *in vitro* studies.

INTESTINAL ABSORPTION OF SUGARS

Absorption of Glucose

Verzar's well known hypothesis[3] that a process of phosphorylation is involved in the rapid absorption of certain of the hexoses from the small intestine has stimulated much investigation and discussion.[17,28–32] Employment of the new *in vitro* technics[23,25,26] has thrown further light on the phenomenon of absorption of sugar by the small intestine. It is clear that glucose not only enters the intestine by simple or passive diffusion but by an active process that is dependent on the normal aerobic metabolism of intestinal tissue, for it is suppressed under anaerobic conditions or by the presence of 2:4 dinitrophenol, a classic example of an agent that dissociates phosphorylations from cell oxidations.[25] Glucose may be transferred against a concentration gradient in the intestine,[26,33] aerobic conditions being essential for the process. A factor that must be considered in work such as this is the conversion of glucose into lactic acid, a process that is marked in the small intestine of the rat, but which occurs only to a small extent in that of the guinea pig.[27,34,35] In any assay of glucose absorption that depends on measurements of differences of concentration of glucose in the mucosal solution, or where the process may be highly sensitive to change of pH, the problem of interference by glycolysis must be considered.

The process by which glucose is absorbed against a concentration gradient or by which it is transported by a mechanism controlled by cell oxidations is termed active transport. This process seems to be unidirectional in the intestine, from the mucosal surface to the serosal. It takes place, in an isolated surviving intestine of the guinea pig, at a constant rate for at least one hour, under optimal aerobic conditions, when examined immediately after sacrifice of the animal. The passive diffusion rate, which can be obtained by examination under anaerobic conditions, is directly proportional to the mucosal concentration of glucose. The rate of active transport of glucose reaches a maximum at a concentration of about 1 per cent, above which higher concentrations of glucose may cause a fall in the rate of active. transport. The relation between the rate of aerobic absorption of glucose and the initial glucose concentration resembles a relation of the Michaelis-Menten type. On plotting reciprocals of aerobic rates of transport of glucose against reciprocals of mucosal concentrations of glucose, as in a Lineweave-Burke analysis, a straight line is obtained[39] giving an *apparent* $K_m = 7 \times 10^{-3}$ M (half-saturation concentration). Fisher and Parsons,[42] in their studies on the effects of glucose concentration in intestinal absorption of the rat, found that the half-saturation concentration of glucose was 8.3×10^{-3} M. This value of an apparent K_m for glucose transport makes it unlikely that hexokinase, if it is involved in the phenomenon of transport of glucose, is rate-limiting, as known

values[43,44] for the K_m of hexokinase of animal tissues for glucose are much smaller than 7×10^{-3} M. In fact it is more reminiscent of the values of the Michaelis constants for phosphatases (e.g., liver glucose-6-phosphatase, $K_m = 2 \times 10^{-3}$).[45]

The process of active absorption of glucose by isolated surviving intestine of the guinea pig is markedly affected by the cation concentrations present in the media bathing the intestine, potassium ions having the largest effect.[39] With a normal potassium ion concentration of 6 mEq./L. the rate of transport of glucose is 36 μ moles/hour; this is increased to 55 μ moles/hour if the potassium ion concentration is increased to 15.6 mEq./L. The higher rate of transport of glucose may be obtained with the normal potassium ion concentration of 6 mEq./L. by doubling the mucosal concentration of glucose. The effect of the potassium ion is simply to bring about an acceleration of the rate at which the maximum velocity of active transfer of glucose is obtained. Potassium ions may be effectively replaced by rubidium but not by cesium ions.

Sodium ions are essential for the process of active transport as their omission and replacement by potassium ions abolishes all active transport of glucose. Small variations of sodium ions from the normal concentration of 122 mEq./L. produce no differences in rates of transport of glucose. Magnesium ions are also necessary for active transport especially for securing the stimulating action of 15.6 mEq./L. potassium ions.[39]

Calcium ions in the media bathing the intestine seem not to be indispensable for active transport, and are often omitted in the experiments *in vitro* as their presence causes very active peristaltic movements. The addition of calcium ions (2.6 mEq./L.) decreases the rate of active transport of glucose by about 30 per cent.[39] Ammonium ions depress the rate of active transport of glucose. Phosphate ions, it may also be mentioned, are without apparent action on transport of glucose,[25] an accelerating effect reported by Magee and Reid[46] being apparently due to the buffering action of the phosphate.[47,48] It is possible that the effects of cations on the active transport of glucose are linked with the active transport of the ions themselves.[85]

Transport of Fructose

The transport of fructose through the intestine is accompanied, under normal aerobic conditions, by conversion of fructose into glucose.[25] This conversion is suppressed by the presence of anaerobic conditions or of 2:4 dinitrophenol. The passive diffusion rate of fructose is not affected by anaerobiosis or by 2:4 dinitrophenol. The so-called active transport rate of fructose is made up of two processes, a passive diffusion rate of fructose together with the transport of glucose obtained by intestinal conversion of fructose.[36] With low concentrations of fructose in the mucosal solution (e.g., 0.02 M) the rate of appearance of glucose in the serosal solution may exceed that of fructose; with high concentrations of fructose the passive diffusion rate increases so that with 0.1 M (or over) the rate of appearance of fructose exceeds that of glucose. In fact, high concentrations of fructose tend to retard the conversion of fructose into glucose.[36] It is of course well known that fructose is converted to glucose in fasting phlorizinised animals[49] and in the liver,[50] a process that occurs *in vivo* in the small intestine,[3,51] but the importance of this transformation in the intestinal transport of fructose only became apparent after these *in vitro* studies.[25,36] D-Fructose is not transported against a concentration gradient in the hamster intestine.[53] The active transport of fructose, as demonstrated by conversion to glucose in the isolated intestine of the guinea pig, is enhanced by the presence of potassium ions (15.6 mEq./L.) but the rate of transport of fructose itself is unaffected by the potassium ion.[39] Recent evidence[88] indicates that conversion of fructose to glucose by intestine of the guinea pig involves rupture of the carbon chain with resynthesis. This does not occur with glucose during transport.[91]

Transport of Other Sugars

Galactose is actively transported by isolated surviving intestine of the guinea pig[25] and in fact the relative absorption rates of glucose, galactose and fructose are similar to those ob-

tained by Cori[2] and Verzar.[3] Its transport is affected by potassium ions in a manner similar to glucose.[39] D-Glucosamine is absorbed at a very feeble rate,[25] a fact confirmed by Sols,[52] and is not transported against a concentration gradient.[53] Mannose and sorbose are also not actively transported.[39,53,54] The presence of potassium ions does not enhance the feeble rates of transport of D-glucosamine, mannose or sorbose.[39] Sugars and related substances transported against a concentration gradient by intestine of the hamster[53] include 3-methyl-D-glucose, 6-deoxy-D-glucose, 1-deoxy-D-glucose, 6-deoxy-D-galactose, D-glucoheptulose besides D-glucose and D-galactose. Sugars and related substances not so transported[53] include 2-deoxy-D-glucose, L-galactose, 1-deoxy-D-mannose, D-xylose, D-ribose, D-arabinose, mannitol, sorbitol and glycerol. Wilson and Crane[53] point out that substitutions at carbon atoms 1 and 6 do not prevent active transport, that compounds which are transported have a D-pyranose structure with a methyl, or substituted methyl group at carbon 5 and a hydroxyl group in the glucose configuration at carbon 2.

Mutual Effects of Sugars on Intestinal Absorption

At concentrations up to 14 mM neither glucose nor fructose affects each other's rate of absorption by the isolated surviving intestine of the guinea pig. Additive rates of transport take place at such concentrations. The rates of absorption of sugars when sucrose alone is present in the lumen of the intestine are those to be expected if the sucrose undergoes hydrolysis by the intestine. The fact that the rate of glucose transport from sucrose is greater than from an equivalent concentration of glucose is due to conversion of fructose derived from sucrose into glucose.[41] At concentrations of glucose higher than 28 mM the rate of sugar transport attains a maximum. Additive effects are not obtained with mixtures of glucose and fructose at or above this concentration. Possibly, inhibition of fructose phosphorylation by glucose[55] or by glucose-6-phosphate[56,57] may be partly responsible for lack of additive effects. Although glucose and galactose are equally well transported in the isolated intestine of the guinea pig, glucose at 14 mM exercises 80 per cent inhibition of galactose transport at an equivalent concentration.[41] Mutual effects of glucose and galactose on their absorptive rates have already been described.[58,59] Sucrose is equally inhibitory but fructose is without effect.[41] Galactose transport, however, is affected by D-glucosamine or by mannose[41] when these are present at equivalent concentrations.

Effects of Inhibitors

Respiratory inhibitors such as cyanide and azide inhibit active transport of sugars through the intestine. Both sodium malonate and fluoracetate and the narcotic chloretone are also inhibitory.[25] The diffusion of sorbose or fructose is unaffected by these inhibitors. Malonate, at a concentration of 2 mM that exerts little inhibition of active glucose transport in the isolated intestine of the guinea pig, brings about over 40 per cent inhibition of glucose transport when this is stimulated by potassium ions. A narcotic such as phenobarbital (10 mM) also abolishes the potassium stimulation.[40] These inhibitory effects of malonate and narcotics, that are markedly increased with potassium-stimulaed transport, are reminiscent of the effects recorded with brain respiration.[60,61,87] The evidence leads to the conclusion that potassium stimulation is associated with an acceleration of the respiratory events of the cell linked with the citric acid cycle, and the effects of malonate, fluoracetate and some narcotics are due to inhibitions of oxidative processes involved in this cycle. The undoubted linkage of active transport of sugars with cell respiration, and the inhibitory effects of 2:4 dinitrophenol,[25,40] which, while not depressing respiration abolishes phosphorylations, makes it evident that phosphorylation processes are either directly or indirectly linked with the mechanism of transport. Neil[81] has reported that 2:4 dinitrophenol (3×10^{-4} M) brings about a loss of intracellular potassium in the intestine of the rat, presumably because of its interference with oxidative phosphorylations. Conceivably the inhibition of active transport by dinitrophenol may be dependent on the changed cation concentration

in the intestine. Triethyltin is also highly inhibitory to transport of glucose in the intestine of the rat, the effect being probably due to inhibition of oxidative phosphorylation.

Phloridzin is an inhibitor of special interest. This substance exerts an inhibitory effect on glucose absorption by the intestine both *in vivo*[15,62] and *in vitro*,[23,33,36] although it will not affect the conversion of fructose into glucose in the intestine.[36] The effect of phloridzin is enhanced with potassium-stimulated glucose transport,[40] a marked inhibition occurring with 10^{-5} M phloridzin. Jervis et al.,[63] confirming the results of earlier workers[15,23,33,36,64] that phloridzin inhibits glucose transport in the intestine, found a marked inhibition with 10^{-5}M phloridzin on the intestine of the rat *in situ*. This phenomenon, however, is complicated by the high rate of glycolysis, in the intestine of the rat, which is also affected by phloridzin.[27] It is suggested that phloridzin prevents entry of glucose into the cell from the mucosal side, a result of which is a diminished rate of glycolysis.[27] The rate of active galactose transport in intestine of the guinea pig is also highly sensitive to phloridzin, a fact pointing to the presence of a common feature in the transport mechanisms of both glucose and galactose. The fact that phloridzin inhibits the rate of fermentation of glucose by acetone-dried yeast[65] and phosphorylations by dry yeast and muscle extracts and glycolysis in brain[15] makes it likely that phloridzin affects the active transport mechanism for both glucose and galactose either by an inhibition of a phosphorylation step or by competition between phloridzin and the sugar for an activating enzyme. It is worthy of note that phloridzin inhibits glucose transport into Ehrlich ascites tumour cells.[71] But this step is either absent, or not rate-limiting, in the conversion of fructose to glucose, a process insensitive to phloridzin. Newey et al.[90] conclude that phloridzin inhibits absorption of glucose by affecting a transfer mechanism on the luminal side of the mucosal cell.

It is clear, however, that phloridzin and 2:4 dinitrophenol do not affect the same mechanism, for the latter blocks conversion of fructose to glucose and also affects active amino acid transport in the intestine which is not affected by phloridzin. Phloridzin affects a specific step concerned with glucose or galactose transport.

As already stated, hexokinase does not control the rate of transport. Mannose undergoes phosphorylation by an extract of rat intestine[55] but is not actively transported. It is known[67] that 1-deoxyglucose and 6-deoxyglucose are actively transported but the constitutions of these molecules make it impossible for phosphorylation by hexokinase to occur. The facts support the hypothesis that separate mechanisms, governing transport of sugars through the intestinal cell membranes, exist. This view is further supported by a new finding[68] that a constituent of croton oil brings about a large inhibition of glucose transport in isolated intestine of the guinea pig with relatively little effect on galactose transport. This fact points to differences between the properties of transporting mechanisms of glucose and galactose. Moreover, the highly inhibitory effect of glucose on galactose transport[41] cannot be due to mutual competition for hexokinase, though inhibitory effects of glucose-phosphate esters on galactose metabolism cannot as yet be ruled out as a possible explanation. The participation of special transport mechanisms in the intestinal cells, a phenomenon now being actively explored in other mammalian cells and in microorganisms, seems to be definite, but with these mechanisms phosphorylations are directly or indirectly connected.

Before leaving this subject, it should be pointed out that phloridzin can cause an inhibition of water transport in the isolated intestine[69] that is accelerated by the presence of glucose.[70] Parsons et al.[27,84] suggest that part of the water movement is dependent on glucose metabolism, the phloridzin affecting water transport by its effects on both glucose transport and metabolism.

TRANSPORT OF AMINO ACIDS

Early work on intestinal absorption of amino acids supported the view that two processes are involved, diffusion[20,73,74] and "active" absorption.[72] L-Alanine is more completely

absorbed than D-alanine[21] and Gibson and Wiseman[75] found in a series of amino acids, using an isolated loop of intestine, that the L-isomer disappears more quickly than the D-isomer. The transport of the L-isomers of alanine, phenylalanine, methionine, histidine and isoleucine takes place against a concentration gradient.[24] The D-isomers are not transported (although D-methionine appears to undergo some active transport[92]), nor are L-glutamate and L-aspartate. However, D-leucine seems to be as actively transported as L-leucine.[38] The L-, and not the D-isomers, of histidine and phenylalanine are transported across the intestinal wall against a concentration gradient, the process being inhibited by cyanide and 2:4 dinitrophenol.[76] Matthews and Smyth[77] found that, when racemic mixtures of phenylalanine and leucine are placed in the lumen of the intestine of the anaesthetised cat, the L-isomer appears in greater quantity than D-isomer in the mesenteric blood, the velocities of transport of the two isomers approaching equality when the temperature is reduced. Active absorption of the L-isomers of alanine, phenylalanine and histidine occurs in the isolated surviving intestine of the guinea pig, such active absorption depending on the respiratory integrity of the tissue.[37] It is suppressed by anaerobiosis or by the presence of 2:4 dinitrophenol, but not by phloridzin.[14–17] Passive diffusion processes, dependent on the amino acid (L or D) concentration in the lumen, take place and become quantitatively more important as the amino acid concentration increases. Although L-glutamate and L-aspartate are not actively absorbed, the corresponding amides L-glutamine and L-asparagine are actively transported, the latter amide (in contrast to L-glutamine) undergoing hydrolysis on passage through the intestine.[37] Active transport of proline, threonine, alanine, glycine, serine, valine, histidine, hydroxyproline, phenylalanine, isoleucine and leucine, takes place in sacs of everted small intestine of the hamster,[78] the transport of proline against a concentration gradient being greatest. Active transport of tryptophan does not occur. The absorption of L-cysteine but not D-cystine is an active process in the intestine of the rat.[81] Wiseman suggests[78] that the mechanism of active transport of amino acids is limited to the mono-amino mono-carboxylic acids (presumably L-glutamine and L-asparagine can be put into this class).

Mutual Effects of Amino Acids on Their Transport in the Isolated Intestine

The L-forms of lysine and ornithine which are not transported actively[78] have no effect on the active transport of proline, glycine, histidine and methionine when present in equivalent concentrations. Pinsky and Geiger[79] showed that L-histidine absorption *in vivo* is decreased by L-tryptophan, and Harper et al.[80] showed that an excess of leucine in the diet inhibits normal utilization of isoleucine. Mono-amino mono-carboxylic acids compete with each other for transport possibly for a common mechanism.[78] New results[82] show that L-methionine, a poorly transported amino acid, at equivalent concentrations inhibits completely the active absorption of alanine and phenylalanine but only about 35 per cent of that of glycine. This result is consistent with a view that these amino acids may have affinities for a common intestinal mechanism but that rates of active transport are not necessarily proportional to the affinities. Hence competitive inhibitions of transport may occur between amino acids just as they may occur between sugars. A cysteine analogue, S-ethylcysteine, a poorly transported amino acid, has a marked inhibitory effect on the active transport of alanine and phenylalanine.[82]

Competition between amino acids for transport into cells is clearly of importance for cell metabolism. Competition between neutral amino acids for reabsorption from the tubular fluids of the dog is stated to occur,[19] and Christensen et al.[66] have shown that when the plasma concentration of amino acids is increased in the guinea pig there is evidence for competition for a cellular amino acid concentrating mechanism. L-Tyrosine, however, seems not to be actively transported in rat liver slices and the presence of L-phenylalanine does not affect tyrosine diffusion, although it inhibits tyrosine metabolism in liver.[83]

Influence of Deoxypyridoxine and Potassium Ions

In view of the possibility that transaminating processes, or other metabolic changes known to affect amino acids, may be involved in the active transport of amino acids, the effects of 0.01 M deoxypyridoxine on alanine absorption from the intestine of the guinea pig were investigated.[37] There were marked inhibitions which were not, however, reversed by pyridoxal, pyridoxine, pyridoxamine and pyridoxal phosphate. This failure to antagonize a deoxypyridoxine inhibition does not necessarily imply that a pyridoxine derivative is not involved in active absorption of amino acids. However deoxypyridoxine also inhibits active absorption of glucose and fructose, indicating a non-specific effect. Whether or not active absorptions of sugars and amino acids are interrelated has yet to be discovered, though it is already known that mutual interferences are possible.[13,37]

Potassium ions have effects on the active transport of amino acids similar to those found with the sugars.[82] Doubtless, the role of energy obtained by cellular respiration is as important for the transport of amino acids in the intestine as it is for the sugars. This is made clear by the results of experiments on the rates of absorption of glucose and amino acids by the isolated surviving intestine of the guinea pig obtained from animals that have succumbed to experimental shock.[38] Intestine from such shocked animals shows, when examined under optimal physiologic conditions, little or no conversion of fructose to glucose, and the rates of active transport of glucose, alanine, leucine (both L and D) and L-glutamine are greatly reduced. The metabolic conditions indeed resemble those obtained by normal intestine under anaerobiosis. Presumably a condition of anoxia is set up in the intestine, during experimental shock, which results in a reduction of active transport mechanisms. It is evident that other pathologic conditions that affect the respiratory and metabolic behaviour of the intestine will also affect the transport mechanisms of sugars and amino acids.

SUMMARY

A short account is given of methods currently in use for the study of intestinal absorption and of the various advantages and shortcomings of these methods. Results obtained in recent studies of intestinal absorption of sugars and amino acids are briefly described. It is pointed out that specific mechanisms responsible for the active transport of these substances exist in the intestine and that competition between one amino acid and another, as well as between one sugar and another, for active transport may take place. Effects of metabolic inhibitors, and cationic changes, on intestinal absorption are described.

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DISCUSSION

Question: What was the effect of insulin on the transfer of glucose or amino acid?

Dr. Quastel: Insulin had no effect *in vitro* on isolated intestine.

Dr. Halvor N. Christensen: I surely regret that Dr. Quastel felt it necessary to state his results so compactly as he did. They are most interesting. I should like to ask one question, with the privilege of commenting on the answer. What level of 4-deoxypyridoxine is effective as an inhibitor of amino acid transport? What level did you use?

Dr. Quastel: I think we used one hundredth molar.

Dr. Christensen: I would like to point out a possibility that you may have thought of as to why pyridoxyl may fail to reverse the action of deoxypyridoxine. We have also observed that deoxypyridoxine is inhibitory. But at an adequate level any of these pyridine compounds becomes inhibitory. And when you further increase their total level—and this will also occur with pyridoxal itself—when you further increase the level in an attempt to reverse an inhibitory effect, you may already have passed beyond a total level where reversion can be obtained.

Dr. Quastel: Yes. I quite appreciate that.

Question: May I ask Dr. Quastel whether he has any information on whether or not the low-molecular-weight peptides, such as glutathione, are absorbed as such?

Dr. Quastel: We have not experimented on this. Other people have worked with the peptides, and they indicate that there is a peptide split. The peptides themselves seem not to be absorbed as such, or only at a very low rate. But there is hydrolysis, and free amino acids are transported.

Question: How did very actively absorbed substances like glutamine rate in the competition? Were they more competitive?

Dr. Quastel: The question is whether in the presence of glutamine one can get evidence of competition? We have not yet carried out this experiment. So far as we have observed, with glutamic acid itself or rather with glutamate, this amino acid does not seem to inhibit transport of other amino acids. But we have not yet tried glutamine.

Dr. D. Mark Hegsted: One question bothers me a little not only in this field but in others. In a case like potassium inhibition, can you ever be sure that sodium is or is not essential if you replace it by potassium? How do you get around this problem when you have to maintain isoosmolar solutions?

Dr. Quastel: You increase the potassium ion concentration and diminish that of sodium.

Dr. Hegsted: And since that is already inhibitory at high levels, is there any way to solve the problem?

Dr. Quastel: Yes. It is possible also to carry out experiments with sodium ions present at normal concentrations and with increasing potassium concentrations. The medium is hypertonic but absorption still takes place. There seems to be no doubt that if sodium is omitted altogether transport is blocked.

Dr. Christensen: Perhaps I could comment on that point, too. At least for transport into cells one can replace the sodium with lithium, and to some degree with tetra-alkyl ammonium ions. There is some latitude, apparently, in this connection.

Dr. Quastel: Yes.

Question: Is there potassium inhibition by increasing your sodium concentration? In other words, by changing the balance of the sodium ion to the potassium ion, does that modify the activity of the potassium?

Dr. Quastel: All that happens, as you increase the sodium but keeping the potassium concentration constant is that you tend to diminish the total rate of transport.

Question: I think you said if you omit the sodium, then you get complete inhibition.

Dr. Quastel: Yes.

Question: But as you increase the sodium, does that change the picture?

Dr. Quastel: Then the solution may become hypertonic. And when the system becomes hypertonic the rate of absorption is affected (see Riklis and Quastel, *Canad. J. Biochem. & Physiol.*, 36:347, 1958).

Metabolic Changes Accompanying the Ingestion of Particulate Matter by Cells

MANFRED L. KARNOVSKY, PH.D.* AND ANTHONY J. SBARRA, PH.D.†

THE WORK to be described in this paper was undertaken as part of a biochemical study of the host-parasite relationship, and might perhaps be considered more germane to a symposium on infectious disease, since the mammalian cells under investigation are leukocytes, and the process studied is phagocytosis. However, if one considers the fact that the engulfment of particulate matter has long been recognized as an important nutritional function, especially in primitive amoeboid organisms, our observations assume relevance in a program devoted to the mechanisms of absorption. The questions investigated are the following:

(1) Does a phagocytic cell expend metabolic energy in accumulating particles? More generally, what is the nature of the metabolic changes that occur during phagocytosis? Experiments concerned with these problems have constituted the major part of our work.

(2) What happens to various particles which are engulfed? Are they rapidly broken down to metabolizable fragments and used by the engulfing cell?

(3) When particles enter a leukocyte, does external medium leak in with the particles, and does this make it possible for the cell to accumulate substances which would otherwise penetrate the cellular membranes slowly or not at all?

The last two questions have received only preliminary attention in our laboratory.

METABOLIC CHANGES ASSOCIATED WITH PHAGOCYTOSIS

The classic theories concerning the mechanism of phagocytosis of Fenn[1] and Ponder,[2] reviewed by Mudd et al.,[3] placed prime emphasis on the surface forces that exist in a system containing phagocytes and particles, i.e., surface forces between cell and particle, cell and medium and particle and medium. Whether or not phagocytosis will occur in a given system was held to be governed by the balance of these forces. Expenditure of metabolically derived energy on the part of the cells was not considered. It has been held by some, e.g., Berry and Spies[4] that expenditure of metabolic energy during the phagocytic event would be unnecessary in terms of the theory proposed by Fenn.

Although considerations of the importance of surface forces, based particularly on the nature of the particle surface, and their role in phagocytosis appear to us to be well founded, a number of metabolic concomitants of phagocytosis would seem to be fundamental to the

From the Biophysical Laboratory and the Departments of Biological Chemistry and of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts.

* Associate Professor of Biological Chemistry, Department of Biological Chemistry and the Biophysical Laboratory; † Research Associate, Department of Bacteriology and Immunology and the Biophysical Laboratory, Harvard Medical School.

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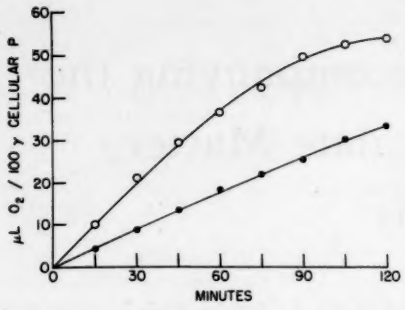

FIG. 1. Increase in respiration of polymorphonuclear leukocytes in the presence of polystyrene spheres. Phase microscopy indicated that these particles were ingested by the cells. ○ = polymorphonuclear leukocytes and polystyrene particles. ● = polymorphonuclear leukocytes alone.

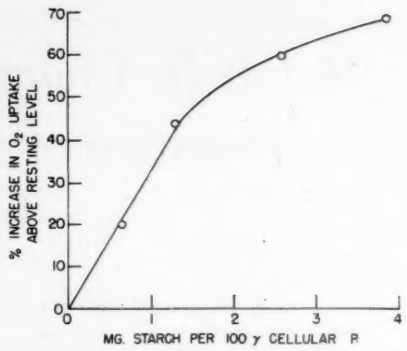

FIG. 2. Relation of the magnitude of the increment in oxygen uptake to the load of particles to which they were exposed. In this case insoluble starch particles were used.

process. For example, when polymorphonuclear leukocytes of the guinea pig are exposed to inert particles, such as bacteria, insoluble starch particles or polystyrene latex particles,* [5–7] there is a great increase in oxygen uptake as the particles are engulfed (Fig. 1). If the effect does indeed represent a metabolic component of the phagocytic process, one might expect the magnitude of the increase in respiration to vary with the load of particles engulfed. An attempt to test this point was made by exposing leukocytes to varying concentrations of particles. The result is shown in Figure 2. It is clear that the more particles present, the greater the respiratory response of the cells. Direct microscopic counts of the number of particles ingested showed that in the range of concentrations used more particles were engulfed when the concentration of particles offered to the cells was increased.

A further indication that the increased oxygen uptake was linked to the phagocytic event was obtained from the effect of serum on the phenomenon. Serum is known to contain factors essential for the optimal uptake of various particles, and the results of exposing polymorphonuclear leukocytes to a standard concentration of particles at various concentrations of serum are shown in Figure 3. Again, correlation of the magnitude of the increase in respiration with microscopic counts was good, and it may be seen that although addition of serum to systems containing no particles ("resting" cells) had only a very small effect, the effect when particles were present was dramatic. Heated serum did not permit this noteworthy effect. Although serum factors are essential for optimal uptake of insoluble starch particles and bacteria, these do not appear necessary for the uptake of polystyrene particles. This observation is construed as indicating the importance of the particle surface with respect to its engulfment and the interrelation of particle surface and factors of the medium in determining whether or not engulfment occurs. The metabolic changes which accompany phagocytosis occur, however, whether or not such factors are necessary for engulfment.

The effect of phagocytosis on the utilization of glucose by the cells was also studied, and the results are shown in Table I. It is clear that under either aerobic or anaerobic conditions, phagocytosis was accompanied by increased production of lactate. Under the latter conditions, utilization of glucose was also increased during particle uptake. It will be shown later (Fig. 4) that direct microscopic counts of ingested particles indicated that

* We are deeply indebted to the Northern Regional Research Laboratory, Peoria, Illinois, for a gift of insoluble Amaranthus cruenthus starch, and to Dr. J. W. Vanderhoff and the Dow Chemical Company, Midland, Michigan, for a generous supply of polystyrene particles. Of the latter the most suitable were spherules 1.171 μ in diameter.

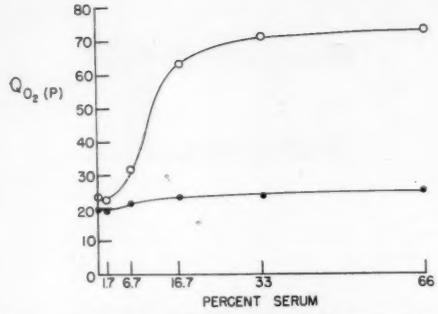

FIG. 3. The effect of serum on the oxygen uptake of polymorphonuclear leukocytes exposed to an excess of starch particles (*upper curve*), and those not exposed to particles (*lower curve*). Q_{O_2} (P) refers to the oxygen uptake in one hour per 100 μg. cellular phosphorus.

TABLE I

Utilization of Glucose and Production of Lactate During Phagocytosis*

Conditions	Particles	Glucose Used† (μM)	Lactate Produced (μM)
Anaerobic	−	2.3	2.8
	+	4.3	3.6
	P‡	<0.02	<0.05
Aerobic	−	1.2	1.9
	+	1.1	2.6
	P‡	<0.9	<0.02

* Results expressed per 100 μg. cellular phosphorus for a thirty-minute period of incubation.

† Including glycogen breakdown.

‡ P has the usual connotation as a probability value referring here to differences due to phagocytosis.

phagocytosis occurred equally effectively under aerobic or anaerobic conditions. A more detailed picture of changes in glucose metabolism during the phagocytic event is given in Table II, which refers to aerobic conditions. It may be noted that oxygen uptake is doubled, and so also is appearance of C-6 of glucose as CO_2 during particle uptake. Conversion of glucose C-1 to CO_2 was, however, increased sevenfold during phagocytosis. Thus, it would

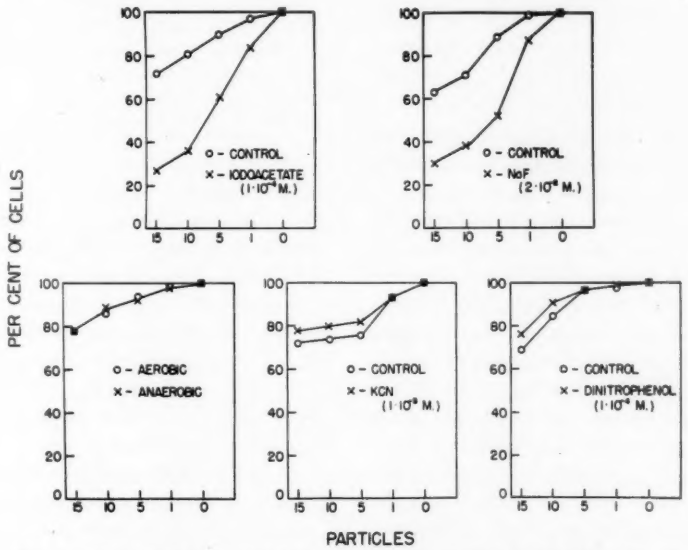

FIG. 4. The effects of anaerobiosis and various metabolic inhibitors on particle uptake by polymorphonuclear leukocytes. The abscissa refers to the number of cells containing the number of particles (polystyrene spherules) indicated, or more than that number. Thus, all cells (100 per cent) contain zero or more particles. Further, for example, in the top left hand panel, 72 per cent of the control cells have ingested fifteen or more particles while only 27 per cent of the cells contain fifteen or more particles in the presence of iodoacetate. The differences from control values in the case of cyanide and dinitrophenol are not thought to be real.

TABLE II

Respiration, and Conversion of Glucose to CO_2 During Phagocytosis*

Particles	Q_{O_2} (P) (μL.)	Activity in CO_2 (counts per minute)		
		C-1†	C-6†	C-1/C-6
−	18.7	211	26	8.1
+	45.6	1406	65	21.6
P	<0.001	<0.001	<0.01	...
Total experiments	34	25		

* All results are given on the basis of 100 μg. cellular phosphorus an hour. Radioactivity data are normalized to 1 × 10^5 counts per minute added per flask.

† Glucose-1-C^{14} or glucose-6-C^{14} as substrate.

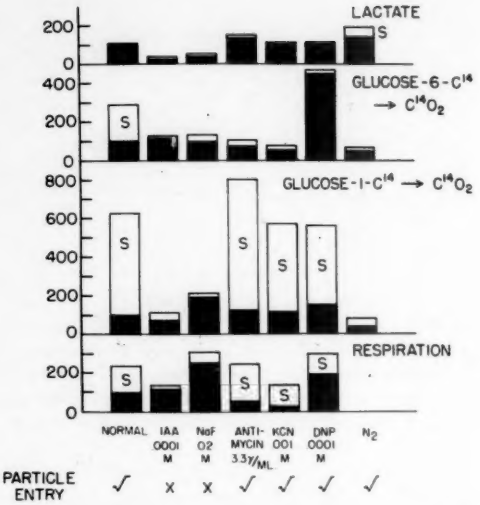

FIG. 5. The effects of inhibitors on metabolism during phagocytosis. The values for normal cells at rest are set at 100. The dark bars refer to "resting" cells under all conditions. The total height of the blocks refers to cells in the presence of particles. Effects on oxygen uptake, conversion of glucose C-1 and C-6 to CO_2, and on production of lactate are given. S refers to differences between "resting" cells and cells exposed to particles which are significant. The entry of particles is referred to as having occurred (√) or not occurred (X) above the minimal level (see also Fig. 4).

appear that utilization of glucose via the direct oxidative pathway (hexose-monophosphate shunt) is greatly stimulated during phagocytosis, relative to the pathway via glycolysis and the tricarboxylic acid cycle.[8]

Effects of Inhibitors of Metabolism

It was thought that a study of the effect of various well known inhibitors of metabolic reactions on phagocytosis and on the metabolic concomitants of the process would be revealing. The results of a number of conditions on particle uptake are shown in Figure 4. It may be noted, as mentioned before, that anaerobiosis has no effect. Inhibitors of glycolysis such as iodoacetate and fluoride sharply depress particle uptake, whereas such compounds as cyanide or antimycin A, which block the cytochrome system, have no effect. Dinitrophenol, which uncouples oxidative phosphorylation, is also without effect. The influence of these substances on the metabolism of "resting" cells and those offered particles may be seen in Figure 5. It is clear that where particles do enter the cells respiration is increased, and the appearance of C-1 of glucose as CO_2 is tremendously accentuated. A point of interest is the fact that fluoride appears to accentuate the direct oxidative pathway for glucose oxidation, and respiration, in "resting" cells, perhaps because more glucose-6-phosphate is available for this pathway when glycolysis is blocked. A further striking feature is the fact that under aerobic conditions increased production of lactate due to phagocytosis was detected only in short term experiments (thirty minutes). If the experiments were protracted for one hour or more, it was found that cells which had phagocytized had produced no more lactate than "resting" cells. It is believed that a situation akin to an oxygen-debt in muscle exists. The tricarboxylic acid cycle and cytochrome system are thought to have a very limited capacity in polymorphonuclear leukocytes of the guinea pig. Under the influence of a rapid demand for energy to accomplish particle engulfment, there is a burst of production of lactate. When phagocytosis is complete, lactate is reutilized as the tricarboxylic acid cycle and the cytochrome system catch up.

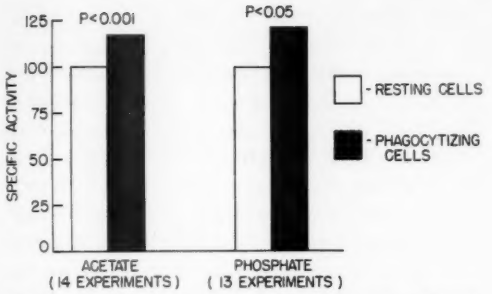

FIG. 6. Effect of phagocytosis on lipogenesis. Experiments were conducted for one hour. Labeled acetate ($CH_3C^{14}OOH$) and inorganic P^{32}-phosphate were the precursors.

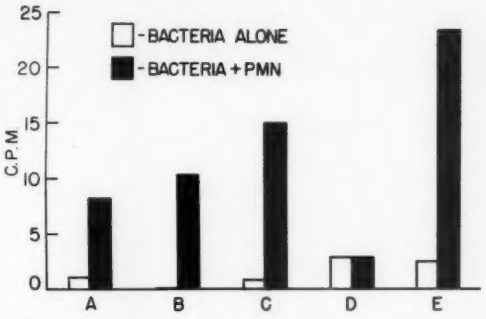

FIG. 7. The digestion and oxidation of bacteria by polymorphonuclear leukocytes. (All values have been calculated to 1×10^8 leukocytes, 1×10^4 counts per minute introduced into the flask as carbon-labeled bacteria, and are given per hour of incubation.) A, Mycobacterium phlei. B, Micrococcus pyogenes. C, Bacillus subtilis. D, Mycobacterium tuberculosis (R_1R_7). E, Mycobacterium tuberculosis (R_1R_7) sonically disrupted.

Possible Functions of the Increased Utilization of Glucose via the Shunt Pathway

From the data on the effect of cyanide and antimycin A, it is clear that transhydrogenase plays little or no part in permitting the reoxidation of reduced triphosphopyridine nucleotide (TPNH) generated in the increased utilization of glucose via the shunt pathway. There is as yet no evidence that direct oxidation of TPNH, not mediated by the cytochrome system, yields cellular energy. The question thus arises as to whether or not a role may be ascribed to the increase in the utilization of glucose via the direct oxidative pathway during phagocytosis. One possibility has been explored to some extent. It has been considered that when particles enter cells they are surrounded by outer cellular membrane. That is, as the particles strike the surface of the leukocyte they may be enveloped by processes of the cell, or carried into a deep invagination by membrane flow. A vesicle containing the particle is then budded off into the cytoplasm. The outer membrane of the cell which surrounds the ingested particles and bounds the vesicle is then disintegrated to leave the particle free in the cytoplasm. These concepts have been discussed by Palade[9] and by Bennett.[10] They would seem to apply in the work of Holter and his colleagues[11] on pinocytosis by amoebas, where droplets of outer medium are engulfed and transported into the cell. Such processes as pinocytosis and phagocytosis, which may not be fundamentally different from each other, are assuming considerable importance in concepts of transport of various substances.

Electron micrographs of our phagocytizing polymorphonuclear leukocytes indicate that phagocytized particles pass into the cells surrounded by membrane.[12] The question, teleologic as it is, might be raised as to whether the cell replaces the membrane ingested with the particles by synthesizing new membrane during, or just after, the phagocytic event, and whether or not the greatly increased production of TPNH might be associated with this accelerated biosynthetic activity.

Since membranes are known to be rich in lipids, particularly phospholipids, an effort was made to investigate the relative extents of incorporation of acetate-1-C^{14} carbon and of phosphate-P^{32} into the lipids of leukocytes at rest and during phagocytosis. Some results are given in Figure 6, and it may be seen that under aerobic conditions, there is an increased lipid synthesis from the labeled building blocks mentioned. The effect was even more dramatic under anaerobic conditions.

These results might give some credence to the ideas expressed concerning the function of the increase in the shunt pathway during phagocytosis. From results in Figure 5 it may be noted that appearance of glucose C-1 during phagocytosis is increased under both

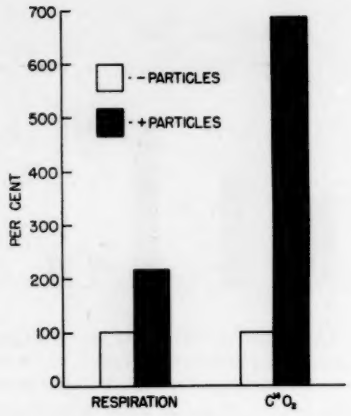

FIG. 8. Effect of ingestion of particles on the "leakage" of medium into the cell. C^{14}-labeled starch was dispersed by gently warming the medium, which was then filtered. Cells were incubated in the medium which contained glucose, with or without polystyrene particles.

aerobic and anaerobic conditions, but as might be expected, to a far lesser degree under the latter circumstances. It might be postulated that TPNH from the shunt pathway is "drained away" under aerobic conditions by a non-cytochrome-linked pathway, and that the direct oxidative pathway for glucose must be stimulated considerably in order to maintain an adequate level of TPNH for biosynthetic purposes.[13] Under anaerobic conditions levels of TPNH would be more easily maintained. The cellular location and exact nature of lipid newly made during phagocytosis are now under detailed study, and constitute questions essential to the argument adduced.

DIGESTION AND UTILIZATION OF INGESTED PARTICLES BY THE CELLS

Although the biochemical mechanism of particle entry has been our main preoccupation, the fate of ingested particles of various types has been of interest, especially where the ingested particles were bacteria. Figure 7 shows results concerning the conversion of the carbon of ingested dead C^{14}-labeled bacteria to $C^{14}O_2$ by leukocytes. It is of great interest that the intact tubercle bacillus, in contrast to some other bacteria, is not rapidly attacked after ingestion, whereas the ingestion of tubercle bacilli lightly sonicated to "crack" them open leads to their rapid digestion and oxidation.[14] Presumably the outer surface of these bacteria is protective against leukocytic enzymes, a supposition not too surprising in view of the composition of these organisms.[15]

PASSAGE OF MEDIUM AND SOLUTES INTO CELLS DURING PHAGOCYTOSIS

Finally, we have made a few studies on the "leakage" of medium into leukocytes with the engulfed particles. Studies are in progress on cation transport by this mechanism, and we have also examined the transport of organic substances which would otherwise not be brought into the cell. Two examples of the latter studies are shown. In Figure 8 the entry of soluble uniformly labeled starch is shown in terms of its intracellular conversion to $C^{14}O_2$. It may be noted that respiration increases a little more than twofold as polystyrene particles are ingested whereas appearance of $C^{14}O_2$ due to degradation of starch

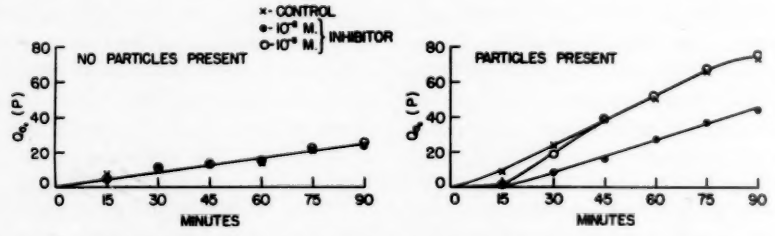

FIG. 9. "Leakage" of an inhibitor into phagocytizing cells. The concentrations of inhibitor refer to the concentrations of glycolaldehyde phosphate which had been treated to form the tetrose diphosphate. Conversion was about 40 per cent, and the actual concentrations of the tetrose diphosphate on a molar basis would thus be about one-fifth the concentrations indicated.

which has presumably entered the cells with the particles increases sevenfold. Figure 9 shows the results of an experiment with an inhibitor of triose-phosphate dehydrogenase, i.e., tetrose-2,4-diphosphate, which does not enter intact cells.* On the left it may be seen that the inhibitor has no effect on the respiration of "resting" cells, since it does not penetrate. On the right one may see that particle ingestion allows the leakage of some of the inhibitor into the cells, resulting in inhibition of further particle uptake and respiration. The effect appears to be overcome at a later time, and in the case of the lower concentration of inhibitor, normal values finally are achieved within an hour. The significance of this interesting recovery phenomenon remains to be evaluated.

CONCLUSIONS

Our present thinking concerning the uptake of particulate matter by cells, at least with respect to polymorphonuclear leukocytes, might be summarized by the following:

(1) Particle uptake is strongly influenced by the nature of the particle surface and factors in the medium, as indicated by classic theories.

(2) The phagocytic event does demand a special energetic output on the part of the engulfing cell.

(3) Glycolysis provides the energy immediately required for phagocytosis and there is an increased production of lactate. Under aerobic conditions some of the lactate produced is oxidized to CO_2 via the tricarboxylic acid cycle and the energy thus provided utilized to return lactate carbon to glucose and glycogen carbon by reversal of glycolysis. Aerobiosis apparently only conserves substrate through more efficient production of energy and is not essential for optimal phagocytosis. From the data on anaerobic phagocytosis and counts of particles ingested, we have calculated that from 1 to 2 $\times$ 10^{-15} moles of high-energy phosphate are required for the engulfment of a polystyrene sphere 1.171 μ in diameter by a polymorphonuclear leukocyte of the guinea pig.

(4) The direct oxidative (shunt) pathway for glucose metabolism is greatly increased during phagocytosis. The greatest fraction of the increase in oxygen uptake associated with particle uptake is not cytochrome linked and is apparently associated with the shunt mechanism.

(5) During phagocytosis there is a significantly increased incorporation of acetate carbon into lipid, which we have considered to be related to the increased availability of TPNH due to the greater passage of glucose through the shunt pathway. Incorporation of phosphate into lipids is also increased. This increased lipogenesis may be due to increased membrane synthesis for replacement of membrane entering the cell as a vesicle around the particles.

(6) Some particles, such as a number of bacteria or particles of starch, are partially digested and oxidized to CO_2 after engulfment. Others, such as the tubercle bacillus are not broken down in these short term experiments presumably because of the resistance of the cell surface to enzymatic attack.

(7) External medium and substances not normally able to enter the cell easily may do so during particle uptake, presumably within the membranous vesicles surrounding the particles, the membrane of which then disintegrates to release the particle and some external medium in the cytoplasm.

*We wish to express gratitude to Dr. E. Racker of the Public Health Research Institute, New York, for a gift of glycolaldehyde phosphate which was converted to the tetrose diphosphate, and for information on the mode of use of this interesting inhibitor.

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DISCUSSION

DR. J. M. QUASTEL: In connection with these very interesting experiments, I would like to ask whether or not Dr. Karnovsky has actually tried to see if during phagocytosis the uptake of radioactive glucose by the leukocyte has increased. I think that during phagocytosis there is an engulfment of fluid containing external solids as well as substances in solution. There is, therefore, formed in the cell an extra concentration of sugar, or maybe other substances, which would be equivalent to an increased rate of transport into the cell. If the cell concentrations of glucose increased, it is conceivable that this increased concentration could stimulate differentially the processes involving glucose-1-C^{14} and glucose-6-C^{14} because of possible differences in the rate-limiting steps and in the respective Michaelis constants in the reactions involved. So the phenomenon of differential stimulation may be simply due to the change in concentration of glucose. I wonder what you think about that.

DR. KARNOVSKY: The question raised by Dr. Quastel is fundamental and of tremendous importance to us, and I am glad that we thought of it very early.

We have not been able to demonstrate any greatly increased transport of glucose into these cells, but I do not think our experiments have been very good yet.

However, we have been able to incubate these cells in many different concentrations of glucose, ranging from rather low C-1 to C-6-labeled glucose concentrations to very high concentrations. Under these conditions of increasing glucose concentration the parameters that I mentioned as being concomitants of phagocytosis change in exactly the opposite way from that observed during phagocytosis. In other words, the C-1 to C-6 ratio in the expired CO_2 diminishes with increasing concentration of glucose. The more glucose you put in the outer medium, and presumably the more that will go into the cells, by diffusion at any rate, the less is the C-1 to C-6 ratio, by a most dramatic factor.

I am confident that the problem is not merely one of the availability of glucose. I think we are quite confident that the results we see are due not simply to getting more glucose in, that is by suddenly opening the gates during phagocytosis and allowing a lot of glucose to stream in, with consequent changes in the metabolic picture. I believe this is not the case. I think we have controlled that.

DR. HALVOR N. CHRISTENSEN: I was going to ask what cellular substance Dr. Karnovsky might suggest that he could be sure was a non-membrane component to serve as a control for this action on fatty acid synthesis. Perhaps every component of the cell is formed 20 per cent faster.

DR. KARNOVSKY: Of course, this is a problem. We have not yet taken this cell apart systematically to find out what the relative increase of all components of the cell might be, which is essential, I think, for our thesis. If the increased lipid synthesis is specific, it would be much more interesting to us. Your point is quite well taken, and I do not know what to suggest. Maybe Dr. Quastel has something he could suggest.

DR. CHRISTENSEN: Perhaps you could separate the mitochondria and study the turnover rate of mitochondrial constituents.

DR. KARNOVSKY: We are deeply involved in this right now. We take resting cells and phagocytizing cells, and at the end of the incubation with radiophosphate, we homogenize the cells and do differential centrifugation to obtain the lipids from each of the cellular fractions. We, of course, do the proteins too—which so far show no increased labeling during phagocytosis. We hope to have the results complete in a year.

DR. D. MARK HEGSTED: Does lipid get through the gut wall in this way?

DR. KARNOVSKY: These polystyrene particles, which are tremendously lipophilic get into not only these leukocytes, but also into some types of ascites tumor cell very rapidly. Judging from what they do, I would think that the possibility is quite good that the microvilli exhibit the formation of vesicles containing lipid, which move inward. This might very well be a pathway.

Some Nutritional Effects on the Metabolism of the Gastrointestinal Mucosa

J. J. VITALE, SC.D.,* D. M. HEGSTED, PH.D.† AND N. ZAMCHECK, M.D.‡

THERE ARE many studies relating nutrition to the pathogenesis of gastrointestinal lesions. Most have been limited in scope and related primarily to gastric secretion or mucosal susceptibility. Studies by Jensen[1] demonstrated the high incidence of gastric ulcers in rats fed a diet low in vitamin A. Zucker, Berg and Zucker[2,3] found that rats maintained on diets deficient in thiamine, pantothenic acid or calcium developed ulcers in the forestomach and antrum. Similar lesions appeared in rats maintained for long periods on diets containing suboptimal amounts of the B complex vitamins.[4] Acute gastric ulcers were found in rats maintained on diets deficient in thiamine[5] and riboflavin.[6] In the latter study, Findlay,[6] observing the similarity of ulcers produced by nutritional deficiencies and inanition, suggested that the latter or some other non-specific affect of nutritional deficiency may be the underlying cause.

The studies of Hoelzel and DaCosta,[7] Matzner and associates,[8] and Weech and Paige,[9] indicated that protein deficiency rather than caloric restriction *per se* may often be the common factor. Diets low in protein resulted in peptic ulcers which were prevented by protein supplements. Hahn et al.[10] produced gastric ulcers in dogs who were protein-depleted.

Nutritional disorders in man are commonly associated with disturbances of the gastrointestinal tract.[11] For example, the gastrointestinal tract is affected in pellagra, beriberi and pernicious anemia, diseases that respond to administration of nicotinic acid, thiamine hydrochloride and vitamin B_{12}, respectively. The diarrhea of sprue may respond to administration of folic acid, citrovorum factor and vitamin B_{12}.[12–14] Ulcerations of the gastrointestinal tract, as well as nausea, vomiting and melena, are associated with the use of folic acid antagonists in cancer therapy and on occasion with antibiotics such as Terramycin® and Aureomycin.®[15]

Even massive gastrointestinal hemorrhages may occur in the course of systemic diseases, in which nutrition and metabolism are impaired.[16] Cox and Barnes[17] produced perforating gastric ulcers in guinea pigs who were injected three times a week with 3 to 6 mg. of histamine. Haverback and associates[18] produced gastric mucosal erosions in the rat following the administration of the serotonin precursor, 5-hydroxy tryptophan. It is interesting to note that 5-hydroxy tryptophan, though producing gastric mucosal erosions, decreased secretion of gastric acid. Gastric erosions have been produced also in experimental animals ingesting alcohol.[19]

A great deal of clinical, as well as experimental, evidence is available to implicate hydrochloric acid in the formation of peptic ulcers.[20] Some emphasis has been placed on the gastric "mucous barrier" although current observations do not appear to support the idea that tissue susceptibility to injury by gastric

From the Department of Nutrition, Harvard School of Public Health, and the Gastrointestinal Research Laboratory, Mallory Institute of Pathology, Boston City Hospital, Boston, Massachusetts.

*Assistant Professor of Nutrition; † Associate Professor of Nutrition, Harvard School of Public Health. ‡ Chief, Gastrointestinal Laboratory, Mallory Institute of Pathology, Boston City Hospital and Clinical Associate in Medicine, Harvard Medical School, Boston, Massachusetts.

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TABLE I

Respiration of Duodenal Mucosa of Pantothenic Acid-Deficient and Control Animals*

Experiment	Deficient Animals		Control Animals	
	Weight (gm.)	Q_{O_2}	Weight (gm.)	Q_{O_2}
1. 90 days	120	6.3 ± 0.57	300	15.5 ± 1.1
2. 42 days	78	6.7 ± 1.1	170‡	4.5 ± 0.7
47† days	...	...	182	4.2 ± 0.8
52 days	...	...	200	11.3 ± 0.8

* From: VITALE, J. J., HEGSTED, D. M., DIGIORGIO, J. and ZAMCHECK, N. *Metabolism*, 2: 367, 1953.[25]

† On the forty-eighth day control animals were fed a diet high in protein and vitamins.

‡ In experiment 2 control animals were fed only 10 gm. per day.

acid is increased as a result of a decreased output or alteration in mucous.[20,21] Emotional disturbances have been shown to influence the secretory and motor functions of the stomach in man and in animals, and in the latter the production of gastric and duodenal ulcers have been demonstrated.[22,23] The problem of elucidating the nutritional requirements for the maintenance of the gastrointestinal mucosa is complicated not only by the multiplicity of nutrients implicated, but by the possible interactions of these nutrients.

The sensitivity of the gastrointestinal mucosa to injury from metabolic changes may perhaps be related to its active metabolism. LeBlond and associates[24] have demonstrated that the gastrointestinal mucosal cells of the rat are regenerated approximately every thirty-six hours. It is not surprising that a tissue that has such a rapid rate of turnover is extremely susceptible to deficiencies or noxious agents of any sort.

Most of the studies that have dealt with the pathogenesis of benign or malignant lesions of the gastrointestinal tract have been morphologic in nature. There has been relatively little effort expended in attempts to sort out the metabolic lesion. The first study we wish to discuss is concerned with the interrelationships between pantothenic acid, protein and calorie intakes on the morphology and metabolism of duodenal mucosa.[25]

TABLE II

Respiration of Duodenal Mucosa of Animals with Pantothenic Acid Deficiency Fed a High Protein Diet and Pantothenic Acid Supplements*

Experiment	Weight (gm.)	Q_{O_2}
1. 65 days	100†	6.2 ± 0.9
2. 30 days	83	14.7 ± 1.1
36 days	77	8.0 ± 1.3
46 days	98‡	11.3 ± 1.0

* From: VITALE, J. J., HEGSTED, D. M., DIGIORGIO, J. and ZAMCHECK, N. *Metabolism*, 2: 367, 1953.[25]

† A high protein-high vitamin diet was fed during the course of this experiment.

‡ Pantothenic acid added (10 mg./kg. of diet) on the thirty-seventh day. Food intake held at previous intake of 7 gm. per day.

PANTOTHENIC ACID

Table I illustrates the respiration of duodenal mucosa of pantothenic acid-deficient and control animals fed *ad libitum;* the Q_{O_2} being 6 and 15, respectively. As in all studies of this kind, there is the question as to whether or not the decreased Q_{O_2} observed in the deficient animals was related to the poor rate of growth or inanition.

Table I also shows the respiration of mucosal tissue from pantothenic acid-deficient and pair fed animals. After forty-two days on the experimental diet, rats with pantothenic acid deficiency had a Q_{O_2} of approximately 6. The control animals at this time also had a decreased Q_{O_2}, approximately 5. Such a drop in respiration might have been expected in deficient animals since their stools gave a positive benzidine test on the thirty-fifth day. No ulcerations were seen in animals who died spontaneously. The stools of control animals, however, gave a negative test. On the forty-eighth day, the control animals were fed a diet containing 60 per cent protein and one in which the vitamin content had been increased five-fold. After four days on this diet, the Q_{O_2} of gastrointestinal mucosa from these semi-starved animals had risen to nearly normal levels, a Q_{O_2} of 11.

Table II illustrates that even with the institution of a high protein and high vitamin diet, but pantothenic acid-free, the Q_{O_2} of mucosa for deficient animals was not signifi-

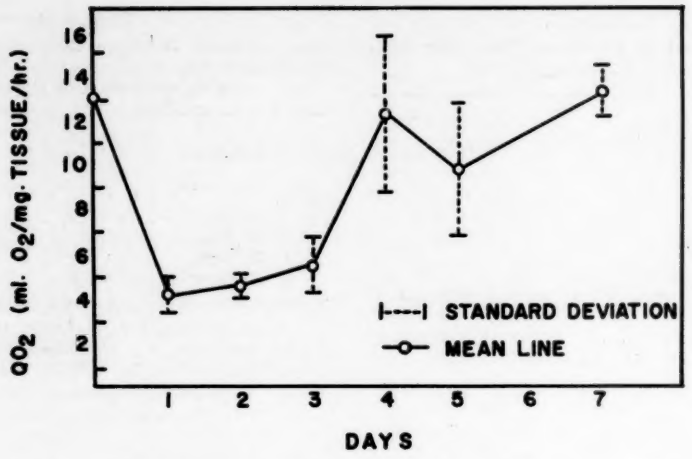

FIG. 1. Effect of aminopterin on duodenal mucosa respiration (dose of 240 μg. per kg. of body weight per day). Each point represents mean of three animals (Figs. 1 through 5 from VITALE, J. J., ZAMCHECK, N., DIGIOVANNA, J. and HEGSTED, D. M. *J. Lab. & Clin. Med.*, 43: 583, 1954[27]).

cantly increased. The addition of pantothenic acid to the diet of deficient animals prevented the decrease in respiration of mucosa (Table II). It is of particular interest that paired feeding or semi-starvation was not an adequate control in this type of experiment. This study demonstrates rather clearly that paired feeding may not provide an appropriate control. Caloric restriction is in itself a complex phenomenon and affects the utilization of other nutrients.

It is desirable to compare the response of two different species. If they are found to respond in a similar manner, one might suspect that the response is a general one and possibly applicable to man. If the response is different, one is provided with additional clues and modes of attacking the problem. Hence,

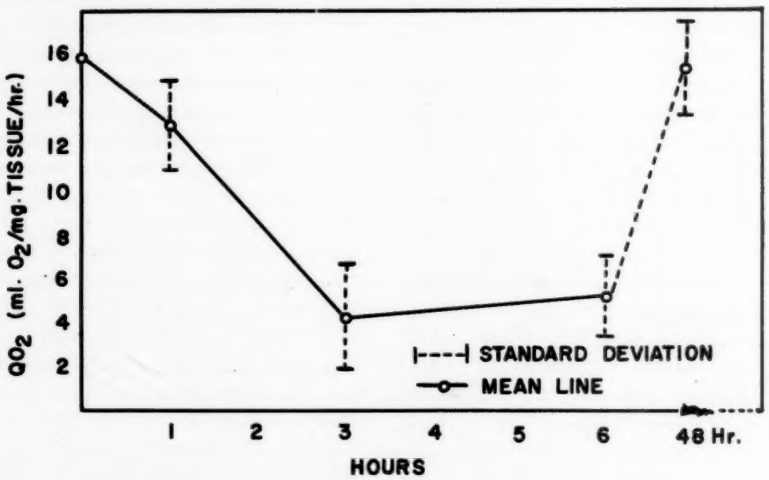

FIG. 2. Effect of aminopterin on duodenal mucosa respiration (single dose of 480 μg. per kg. body weight). Each point represents mean of three animals.

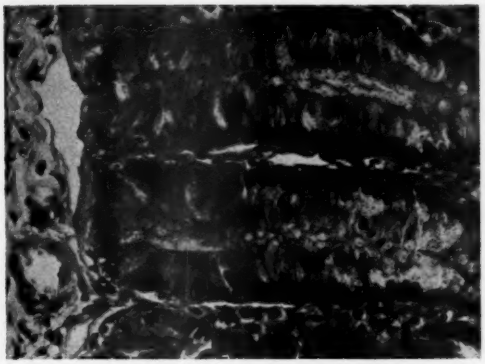

FIG. 3. Normal intestine showing abundant mitoses in duodenal crypts (lumen of crypts to right) (×800; reduced 1/6).

FIG. 5. Complete loss of normal structure. Rat given 120 μg. per kg. daily and sacrificed when terminal on tenth day (×30; reduced 1/4).

we have compared rats and guinea pigs in several of our studies.[26,27]

AMINOPTERIN AND FOLIC ACID

Figure 1 shows the effect of administration of aminopterin on the respiration of mucosal tissue of rats given 240 μg. per kg. of body weight per day. Each point represents the mean of three animals. At the end of twenty-four hours, there was a marked reduction in respiration of this tissue. On the fourth and following days, respiration appeared to return to control levels but a great deal more variation around the mean values was observed. The guinea pig, on the other hand, showed no such change in respiration after similar doses of aminopterin.

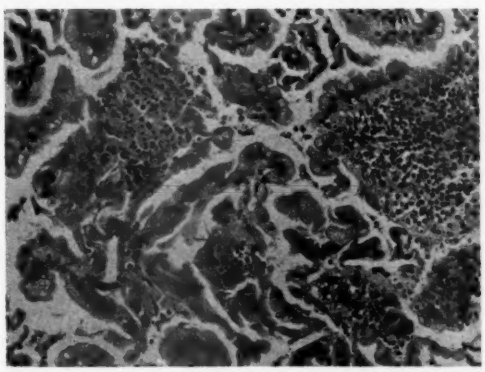

FIG. 4. Duodenal mucosal scrapings showing effect of aminopterin. Distorted mucosal cells, dense tunica propria infiltrated with round cells (240 μg. per kg. daily; sacrificed fourth day) (×400; reduced 1/6).

It would not have been possible to interpret this increase in respiration of rat mucosa after the fourth day without morphologic studies. At the end of twenty-four hours, the only change seen morphologically was a marked reduction in the mitotic activity in the crypts. However, on the fourth and following days, the tunica propria of the duodenum was heavily infiltrated with polymorphonuclear leukocytes which tended to be clustered in large numbers in the crypts. The epithelium was thinned and elongated and the nuclei varied in size and shape. Mitoses in the crypts were virtually absent. It was apparent that we were measuring the respiration not only of epithelium, normal and damaged, on the fourth and following days, but also of white cells which respire actively and of connective tissue which is metabolically inactive.

Figure 2 illustrates the rapidity to which the mucosa responds to a single injection of aminopterin. These animals were given one dose of aminopterin, 480 μg. per kg. of body weight. After three hours, there was a significant drop in respiration. However, after forty-eight hours the respiration had returned to normal levels. The only morphologic change seen was a marked reduction in mitotic activity at the end of three hours. No pathologic lesions were seen forty-eight hours later indicating the rapid regenerative power of gastrointestinal mucosa.

Figure 3 shows normal intestine with abundant mitosis in the duodenal crypts. Figure 4

TABLE III

Effect of Aminopterin on Folic and Folinic Acid Concentrations of Liver and Small Intestine*

Animal	Folic Acid		Folinic Acid	
	Liver	Small Intestine	Liver	Small Intestine
Control				
Guinea pig (10)	1531 ± 390†	48 ± 9	181 ± 47	8 ± 2
Rat (10)	3051 ± 220	384 ± 59	890 ± 220	161 ± 18
Treated with Aminopterin				
Guinea pig (6)	...	...	190 ± 64	6 ± 1
Rat (6)	...	...	891 ± 156	38 ± 11

* From: VITALE, J. J., GERSHOFF, S. N., SINISTERRA, L., HEGSTED, D. M. and ZAMCHECK, N. *J. Biol. Chem.*, 220: 363, 1956.[26]

†μμg./gm.

demonstrates duodenal mucosal scrapings from an animal treated with aminopterin and sacrificed on the fourth day. Mucosal cells are distorted and the tunica propria is infiltrated with round cells. Figure 5 shows the complete loss of all epithelial structure in terminal animals.

Guinea pigs, however, showed no such changes either metabolically or morphologically under the conditions of these experiments. The Q_{O_2} of intestinal mucosa of the guinea pig was approximately 14. Doses of up to 2,000 μg. per kg. body weight or approximately ten times the dose given to rats failed to produce any changes in the guinea pig. The reason for this may be related to the data shown in Table III, which illustrates the folic and folinic acid content of liver and intestinal mucosa of control and aminopterin-treated rats and guinea pigs. The folic acid concentration in the liver of guinea pigs was approximately 1,500 μμg. per gm. and 48 in the small intestine, whereas the rat had essentially three times as much folic acid in its liver and ten times as much in the small intestine. The folinic acid concentrations in the guinea pig's small intestine was extremely low, about 8 μμg. per gm.

TABLE IV

Specific Activity of Phosphoprotein Obtained from Intestinal Mucosa of the Guinea Pig and Rat*

Segments of Small Intestine	Animal	Two Hours
Duodenum	Guinea pig	1,157
	Rat	11,893
Jejunum	Guinea pig	989
	Rat	6,570
Ileum	Guinea pig	1,212
	Rat	10,659

* From: VITALE, J. J., GERSHOFF, S. N., SINISTERRA, L., HEGSTED, D. M. and ZAMCHECK, N. *J. Biol. Chem.*, 220: 363, 1956.[26]

compared to the high level of 161 μμg. per gm. of intestine of the rat. Similarly, the rat's liver contained approximately eight times the concentration found in guinea pig's liver. However, administration of aminopterin did not affect the already low concentration of folinic acid of mucosa of the guinea pig but greatly decreased the concentration in mucosa of the rat.

Folinic acid, which is presumably the active form of folic acid, has been implicated in many enzyme systems, notably those involved in purine and pyrimidine synthesis. Paterson and Zbarsky[28] found that the gastrointestinal mucosa incorporates glycine and formate, at very high rates, into desoxyribose nucleic acid and ribonucleic acid. This is probably related to the high mitotic activity or regeneration of mucosal cells. Thus, one might suspect a slower turnover rate of gastrointestinal mucosa of guinea pigs as compared to rats. Using the uptake of labeled phosphorus as a measure of mitotic activity or rate of turnover, the results expressed in Table IV would indicate that the cells of intestinal mucosa of the rat proliferate at a far greater rate than those of the guinea pig. These values represent P^{32} activity of phosphoproteins. At the end of two hours, the specific activity of the phosphoprotein obtained from the mucosa of the duodenum, jejunum and ileum of rats was approximately 12,000, 10,000 and 6,000, respectively, compared to 1,000, 900 and 1,200 for similar sec-

TABLE V

Succinoxidase and Xanthine Oxidase of Intestinal Mucosa Homogenate of the Rat and Guinea Pig*

Animal	No. of Animals	Succinoxidase [Q_{O_2} (N)]	Xanthine Oxidase [Q_{O_2} (N)]
Rat	37	<10	
	8	50–100	
	5	100–210	
	3		12 ± 0.16†
Guinea pig	5	313 ± 18†	12 ± 0.21†

* From: NAKAMURA, M., PICHETTE, P., BROITMAN, S., BEZMAN, A. L., ZAMCHECK, N. and VITALE, J. J. *J. Biol. Chem.*, 234: 206, 1959.[29]

† Standard error.

tions of the gastrointestinal tract of guinea pigs.

ENZYMES

When studies on specific enzymes were begun, succinic oxidase activity was the first enzyme system selected. In many attempts to study this system in gastrointestinal mucosal homogenates of the rat,[29] we failed although, histochemically, succinic dehydrogenase was always present in intestinal mucosa of the rat. Frustrated by the fact that we were only able to measure little and at times no activity in homogenates of the rat, we turned to intestinal homogenates of the guinea pig and found that we could get consistent and reproducible results. In sixty-seven experiments, the average Q_{O_2} (N) of homogenates of the rat was less than 10. In eight animals, the Q_{O_2} (N) varied between 50 and 100 and in five rats, between 100 and 200. In guinea pigs the Q_{O_2} (N) of mucosal homogenates varied between 400 to 500. There was no difference in xanthine oxidase activity between intestinal mucosal homogenates of the rat or guinea pig, both having a Q_{O_2} (N) of approximately 12 (Table V). Furthermore, the addition of mucosa of the rat to mucosal homogenates of the guinea pig always depressed the oxidation of succinate. Two papers have appeared in the Italian literature[30,31] demonstrating that the addition of serum to homogenates of rat's heart increased the oxidation of succinate by this tissue.

Table VI shows the effect of adding serum to homogenates of rat's gut (Table VI). The addition of serum in each case increased the oxidation of succinate. In this experiment, in addition to testing the effect of serum on succinate oxidation, the inhibitory effect of the same homogenate of rat's gut was also tested on succinic oxidase activity of the rat's heart. It is obvious from these data (Table VI) that when the activity of the homogenate of rat's gut was low, the effect of serum was great and maximum inhibition of heart activity was observed. Conversely, when mucosal homogenates of the rat showed appreciable activity, the effect of serum was decreased and less inhibition of heart activity was seen. It appeared that we were dealing with an inhibitor and that the effect of serum was to "bind" this inhibitor. The addition of human or bovine albumin, rather than serum, produced the same results. Apparently, the active component of the serum was the protein. No inhibition could be demonstrated in intestinal mucosal homogenates of the guinea pig.

TABLE VI

Relationships between Rate of Inhibition of Succinoxidase of Rat Heart by Intestinal Mucosa of Rat and Succinoxidase Activity of Intestinal Mucosa of Rat; Effect of Rat Serum on Intestinal Succinoxidase Activity of Rat*

Experiment	Succinoxidase Activity of Rat Intestinal Mucosa [Q_{O_2} (N)]		Inhibition of Heart Succinoxidase (%)
	Without Serum	With Serum	
1	20	120	>90
2	10	60	>90
3	1	22	>90
4	1	100	>90
5	10	40	>90
6	86	183	<20
7	95	230	<20
8	77	183	<20
9	70	155	<20
10	15	30	30
11	104	187	<10
12	98	228	0

* From: NAKAMURA, M., PICHETTE, P., BROITMAN, S., BEZMAN, A. L., ZAMCHECK, N. and VITALE, J. J. *J. Biol. Chem.*, 234: 206, 1959.[29]

TABLE VII

Effect of Human Gastrointestinal Mucosa on Succinoxidase Activity [Q_{O_2} (N)] of Homogenates of Rat Heart*

Human Tissue	Succinoxidase Activity [Q_{O_2} (N)]			
	Serum		Rat Heart Alone	Human Mucosa and Rat Heart
	Minus	Plus		
Stomach	491	449	736	1,043
	301	388	599	695
Jejunum	78	72	514	756
Ileum	140	210	802	768
Colon	161	194	603	741
	266	222	602	1,067
	107	85	602	934

* From: NAKAMURA, M., PICHETTE, P., BROITMAN, S., BEZMAN, A. L., ZAMCHECK, N. and VITALE, J. J. *J. Biol. Chem.*, 234:206, 1959.[29]

Human gastrointestinal tissue obtained at operation at the Boston City Hospital was also assayed for succinic oxidase activity. Two stomachs, one jejunum and one ileum and three colons, morphologically normal, were assayed. Table VII shows the results we obtained with these specimens. Human tissue, in our limited studies, behaved like mucosa of the guinea pig. There was no apparent inhibition of activity of rat heart by homogenates of any of these tissues with the possible exception of the ileum, but there was some increase in activity of this tissue upon the addition of rat serum.

The inhibitor was extracted from mucosal homogenates of the rat by acetone. The acetone filtrate was separated into various components on a silica acid column according to the methods described by Borgström.[32] Of the components thus separated, only the unesterified fatty acids inhibited succinic oxidase activity of homogenates of the rat's heart. Acetone filtrates of intestinal mucosal homogenates of the guinea pig similarly prepared had no inhibitory effect and we were not able to detect any appreciable amount of unesterified fatty acids in mucosa of the guinea pig.

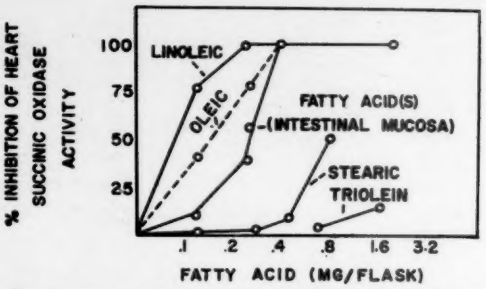

FIG. 6. A comparison of the inhibitory effects of the fatty acids extracted from mucosa of the rat's gut and of pure neutralized fatty acids. The homogenate of the rat's heart contained 0.427 mg. of nitrogen per flask. (From: NAKAMURA, M., PICHETTE, P., BROITMAN, S., BEZMAN, A. L., ZAMCHECK, N. and VITALE, J. J. *J. Biol. Chem.*, 234:206, 1959.[29])

FATTY ACIDS

Figure 6 shows the inhibitory effect of various fatty acids and fats as well as the effect of those extracted from intestine of the rat. The iodine number of these fatty acids of gut of the rat was approximately 110. The inhibitory effects of unsaturated fatty acids on succinic oxidase activity have been described by Edwards and Ball.[33] Stearic acid, unlike the unsaturated fatty acids, inhibited slightly and then at relatively high concentrations. Triolein caused little inhibition. The concentrations of these fatty acids extracted from mucosa of the rat varied from 0.16 to 0.24 mg. per mg. of nitrogen, whereas the mucosa of the guinea pig had concentrations of approximately 0.005 mg. per mg. The difference in activity of the mucosa of the rat and guinea pig is thus related to the free fatty acids present.

There is some evidence that these fatty acids may be associated with the mitochondria of the cell. Mitochondria isolated from intestinal mucosa of the rat failed to oxidize or phosphorylate either succinate or alpha-ketoglutarate. On the other hand, in three different experiments with mitochondria prepared from mucosa of the guinea pig, P : O ratios of 0.98 and 3.15 were obtained in the presence of succinate and alpha-ketoglutarate, respectively. The fatty acids from mucosa of the rat completely inhibited oxidative phosphorylation by mitochondria from intestinal mucosa of the guinea pig.

MAGNESIUM

In studies dealing with the role of dietary magnesium in atherogenesis, we have observed that in the guinea pig with magnesium deficiency, whose requirement for magnesium is approximately twenty-five times that of the rat,[34,35] severe gastric erosions developed which caused fatal massive gastrointestinal hemorrhage in some cases. The rat, on the other hand, is not as sensitive to magnesium deficiency and gastric bleeding is less severe.

In order to produce experimental atherosclerosis a hypercholesterolemia must be induced. In the rat this can be done by the addition of cholesterol and cholic acid to the diet. Recently, we have demonstrated that dietary cholesterol and cholic acid increased the requirement for dietary magnesium.[40] The lipid deposited in the left ventricular valves and aorta of rats could be markedly reduced by feeding large amounts of magnesium. The addition of equal amounts of cholesterol and cholic acid to the diet of guinea pigs results in serum cholesterol levels of approximately three to four times those observed in the rat. By omitting cholic acid from the diet of the guinea pig and feeding only cholesterol, serum cholesterol levels, comparable to those seen in the rat, are obtained. Even so, at equal serum cholesterol levels the amount of lipid deposited in the left ventricular valves and aorta of the guinea pig is approximately ten times that seen in the rat. This may be in part related to differences in their absorption rates of steroids from the lumen and differences in the rate at which these steroids enter the general circulation. Both morphologic and biochemical differences in the mucosa may be involved. We have been able to show much more rapid rates of absorption of xylose in guinea pigs than in rats. In addition to perhaps faster rates of steroid absorption, the vascular system of the guinea pig is also more susceptible to atherosclerosis.

The administration of thyroxine also increases the requirement for dietary magnesium in both *in vitro* and *in vivo* studies.[36–39] The gastric erosions seen in animals with magnesium deficiency can be aggravated by the administration of thyroxine. Interrelationships between thyroxine and magnesium, as well as vitamin B_{12}, have been observed.[41] Table VIII illustrates some effects of dietary vitamin B_{12} and magnesium in animals treated with thyroxine on the vitamin B_{12} levels of liver and intestinal mucosa. The lowered tissue levels of vitamin B_{12} due to administration of thyroxine could be significantly raised not only by administration of vitamin B_{12} but by dietary magnesium as well.

TABLE VIII

Effect of Thyroxine, Magnesium (Mg^{++}) and Vitamin B_{12} on Tissue Vitamin B_{12} Content*

Dietary	Vitamin B_{12} Content ($\mu\mu$g./gm.)	
	Liver	Small Intestine
Control diet plus		
20 mg. % Mg^{++}	54 ± 8	71 ± 7
0.002 mg. % vitamin B_{12}	90 ± 8	97 ± 8
20 mg. % Mg^{++} plus 2 mg. % thyroxine	29 ± 5	44 ± 6
160 mg. % Mg^{++} plus thyroxine	49 ± 7	64 ± 6
0.002 mg. % vitamin B_{12}, 160 mg. % Mg^{++} plus thyroxine	131 ± 16	100 ± 11

* From: GERSHOFF, S. N., VITALE, J. J., ANTONOWICZ, I., NAKAMURA, M. and HELLERSTEIN, E. E. *J. Biol. Chem.*, 231: 849, 1958.[41]

CLINICAL ASPECTS

Up to now, we have looked at one side of gastrointestinal metabolism; namely, the effect of nutritional deficiencies on mucosal metabolism. Perhaps equally important is the role of the gastrointestinal mucosa in the pathogenesis or modification of other disease processes.

Gastrointestinal malabsorptive syndromes and pernicious anemia are other examples of gastrointestinal involvement in systemic diseases. Mayer and Yannoni[42] found increased rates of glucose absorption in mice with experimentally induced obesity. Likoff et al.[43] observed increased rates of absorption of fat in patients with coronary heart disease compared to control patients. These differences in absorption of fat were magnified in those

patients with coronary heart disease and hypercholesterolemia. Pinsky and Geiger[44] demonstrated alterations in the absorption rates of individual amino acids in the presence of mixtures of amino acids, and suggested that the biologic value of proteins may in part be related to absorption phenomena. The implications one can draw from such limited studies are obvious. Comparative studies on gastrointestinal metabolism both with different species of animals as well as in various disease syndromes may be rewarding.

CONCLUSIONS

The relation of gastrointestinal function to nutrition, and vice versa, provides an example par excellence of the need for a better understanding of interrelationships between nutrition and disease. This is becoming more clearly recognized as the major need in nutrition. The task is to define and quantitate the numerous interactions and interrelationships between nutrients themselves as well as the effects they produce. Sufficient examples are now available to demonstrate that one nutrient in deficiency or excess may have large effects upon the body's need or the metabolism of another nutrient. The gastrointestinal tract is extremely sensitive to nutritional and metabolic imbalance and because of this, as well as its anatomic position and function, may be the first determinant in nutrition.

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Classification of Malabsorption Syndrome

Introductory Remarks

DAVID ADLERSBERG, M.D.*

IT IS advisable to classify the malabsorption syndrome into primary and secondary forms. The primary malabsorption syndrome includes several classic entities, celiac disease of childhood and, in the adult, tropical and non-tropical forms of sprue. The primary malabsorption syndrome must be strictly separated from the secondary malabsorption syndrome which is caused by close involvement of the small bowel by such diseases as lymphosarcoma, amyloidosis and jejunoileitis, or resulting from extensive resection of the small bowel, inadvertent gastroileostomy or from liver-pancreatic disease.

The primary malabsorption syndrome, according to our present thinking, is a genetically-transmitted complex metabolic disorder. From the clinical aspect, it is mainly characterized by intestinal malabsorption and hematologic abnormalities. It involves, however, many other metabolic disturbances and affects the metabolism of proteins, carbohydrates, lipids, electrolytes and water and may be hematopoietic, which is not necessarily related to the difficulties in absorption. Profound disturbances in genetically-controlled enzymatic chain reactions seem to affect intestinal absorption mainly in the wall of the gut as well as the metabolic pathways of many factors mentioned above.

Based on the genetic anlage, the malabsorption syndrome may become manifest at various periods of life: in infancy or childhood (as celiac disease) or later in life as tropical or non-tropical sprue. Having produced manifestations in childhood, the disorder may go underground for many years or may never again produce manifestations of malabsorption. As in many other hereditable metabolic errors, there is a steady interplay of the genetic predisposition with environmental influences which may both stimulate and mitigate the manifestations of the disorder. Distress of tropical climates, malnutrition and infections are important environmental factors which may convert a predisposed person into a patient with manifest malabsorption syndrome. Strong emotional factors may play a similar part. Whether wheat or rye gluten represents a dietary triggering mechanism or is more profoundly involved in the basic metabolic defect (pterylglutamate complex) of primary malabsorption remains to be established.

In our climate, we are mostly concerned with one clinical entity of the primary malabsorption syndrome: non-tropical sprue, which will be the main topic of discussion. Reference will be made to the tropical variety of sprue and to the celiac syndrome of childhood.

From the Department of Nutrition, The Mount Sinai Hospital, New York, New York.

* Deceased

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Malabsorption Syndrome: Pathogenesis

W. Trevor Cooke, M.D., F.R.C.P.*

The term "malabsorption syndrome" has attained great popularity since it was first introduced by Cross et al. in 1953.[1] Although it excludes from consideration one of the more common conditions of poor absorption, pernicious anaemia, it has directed attention to the small intestine, a poorly studied area of the body. For reasons which I have given elsewhere,[2] the term is misleading. The common factor between the various conditions is the presence of excess fat in the stools, a sign which is pathognomonic of disturbed function of the small intestine.

It is by no means certain, however, that some types of disorder included under the term malabsorption syndrome necessarily have steatorrhoea as a constant feature. These can be categorised into three groups: (1) those in which there is a disturbance of the digestive processes within the intestinal lumen, (2) those in which the intestinal wall is involved in defined pathologic processes and (3) those in which constitutional and systemic factors appear to be involved. It excludes patients with jaundice and acute diarrhoeal disorders.

The first group is that in which the processes of digestion are disturbed. The simplest disturbance of digestion is pancreatic dysfunction leading to an increased loss of fat and nitrogen in the faeces but not necessarily to disability; it is not associated with any haematologic abnormality and provided intake is adequate, there is no systemic upset of protein metabolism.

More complicated is the clinical picture of marked malnutrition, anaemia and steatorrhoea produced in some patients following gastrectomy. Among the factors concerned here is the mechanical factor of an inadequate stoma or afferent loop, which is too long, leading to insufficient mixing of the pancreatic and duodenal juices with food. In others an associated pancreatic disorder may exist while there are still unknown factors produced by the stomach which are necessary for normal digestion and absorption of fat and protein.

Another important factor is upset in iron absorption, which is slightly impaired following gastrectomy,[3] and subsequently the total body stores are gradually depleted over the years, the time needed varying from one case to another from one to many years. Although it has been claimed that chronic intestinal blood loss is the major cause in these cases[4] this seems to be unlikely except in the occasional patient. It is also to be expected that with resection of a portion of the stomach vitamin B_{12} deficiencies may become apparent. In some patients this will be due to loss of intrinsic factor. It has, however, been shown by Witts[5] and his colleagues in Oxford that the experimentally produced iron deficiency in rats leads to low serum B_{12} levels and impaired absorption of vitamin B_{12} which can be corrected by the administration of iron alone. This same phenomenon can take place in man (Fig. 1).

This observation of course is not new. Hartfall and Witts[6] suggested it in 1932 and the literature contains many observations on iron deficiency anaemia preceding the development of pernicious anaemia. It is not yet clear, however, whether this vitamin B_{12} deficiency is due to the temporary suppression of intrinsic factor or to the effect of chronic iron deficiency on the mucosal cells in the distal portion of the small intestine. It seems probable that either may take place. In the investigation of vitamin B_{12} absorption after gastrectomy reported by Lowenstein,[7] it is worth noting that the only patients who did not absorb vitamin B_{12} were three with severe

From The General Hospital, Birmingham, England.
* Physician and Reader in Medicine.

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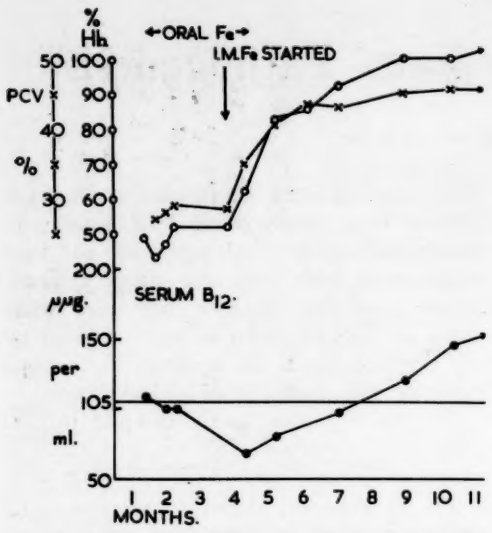

FIG. 1. A fifty year old man. Four years after gastrectomy there is a lack of effect of oral iron therapy with decreasing serum vitamin B_{12} levels and restoration of normal blood picture and vitamin B_{12} levels with intramuscular iron therapy.

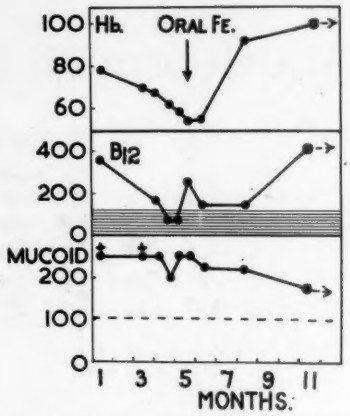

FIG. 2. An eighteen year old boy with regional enteritis. Note the effect of oral iron therapy on the serum levels of vitamin B_{12}. Serum iron at the start of therapy was 26 μg. per 100 ml. Levels maintained subsequently for eighteen months on oral iron therapy.

hypochromic anaemia. It also remains to be shown just what part chronic iron deficiency may play in aggravating the degree of steatorrhoea through changes in the cellular metabolism of the ileum.

Changes in the intestinal milieu may also be brought about by changes in the intestinal flora. In particular this applies to the gastro-jejunocolic group in which the small intestine is continually soiled by colonic bacteria. In this group, the degree of steatorrhoea can be considerably lessened temporarily by the administration of antibiotics. To a lesser extent, the same phenomenon is seen in the entero-enterostomies lower down the bowel. Whether the same considerations hold good in intestinal diverticulae is not so clear. Girdwood[8] has claimed that bacteria isolated from such diverticulae produce significant amounts of folic acid. There is certainly no conclusive evidence that folic acid deficiency is ever produced in such cases, although patients in both these last two groups become deficient in vitamin B_{12} readily. It has been assumed that intestinal bacteria utilise the available vitamin B_{12} in preference to the host.[9]

The administration of antibiotics certainly cures the anaemia. It remains to be proved that this is direct action on the bacteria and not a systemic effect, since it has been observed that some antibiotics which are poorly absorbed do not have this effect.[9,10]

The second group of conditions are those in which the wall of the bowel is affected, such as regional enteritis, enterocolitis, tuberculosis, amyloid and carcinoma. As an example, regional enteritis may be considered. The clinical picture produced will depend upon the area of the gut involved. Some patients may simulate a primary malabsorptive state, as in extensive jejunoileitis. Jejunitis may exist without any significant increase in fat excretion while lesions and resections of the lower part of the ileum are associated with a degree of steatorrhoea. This is somewhat contrary to the findings of Börgstrom and his colleagues.[11]

The defects that I wish to consider particularly are those of iron, vitamin B_{12} and folic acid. Little attention has been paid to the incidence of iron deficiency in regional enteritis but it is high, and in a consecutive series of thirty-nine patients who had not had recent treatment with iron, the mean level was 51.3 per cent, range 12 to 107 μg./100 ml. To some extent this low level varies inversely with the degree of activity

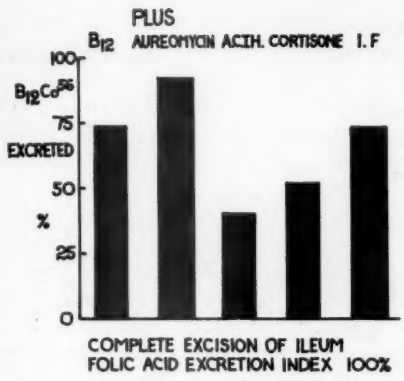

FIG. 3. Note the effects of therapy with aureomycin, ACTH, cortisone and intrinsic factor on the faecal excretion of radioactive vitamin B_{12} in a seventy-five year old man with excision of the ileum for regional enteritis.

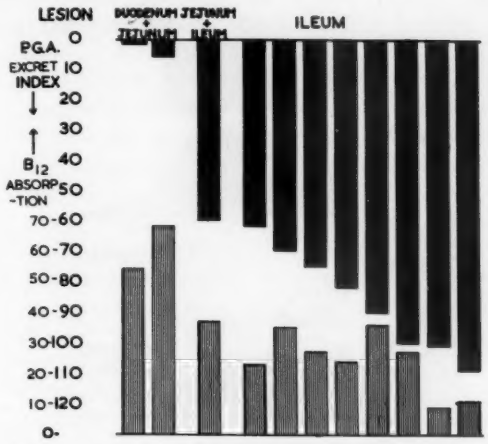

FIG. 4. Data on eleven patients with regional enteritis arranged according to the area of bowel involved. There was good absorption of vitamin B_{12} in the upper portion of the small intestine and poor absorption in the lower portion. At the same time there was a poor response to the folic acid excretion test in patients with jejunal involvement and a normal response when the ileum was involved or resected.

of the intestinal lesion. It may be partially due to the upsets in iron metabolism associated with chronic infection but in most patients it is probably due to chronic blood loss from the intestinal lesion. In one such patient under treatment without effect upon the haemoglobin (about 60 per cent) the average daily loss was 30 ml. of blood. Such chronic iron loss can have an effect upon the serum levels of vitamin B_{12} as is demonstrated in this patient (Fig. 2). However, the incidence of poor absorption of vitamin B_{12} (between 50 and 60 per cent) in chronic regional enteritis is too great to be accounted for by this mechanism alone. The deficiency in some patients may be accounted for by the actual length of intestine resected but such instances are few; in the majority it would appear to be due to involvement of the ileum with either macroscopic or microscopic lesions. Absorption is not improved by the administration of intrinsic factor or antibiotics but it is improved by the administration of steroids (Fig. 3).

Actual folic acid deficiency is rare in regional enteritis. Tissue unsaturation without clinical manifestations may occur through pure dietary inadequacy but actual haematological manifestations will only be found when the upper part of the jejunum is involved. By use of the folic acid excretion test[12,13] it can be shown that the site of absorption for folic acid is in the upper portion of the intestine (Fig. 4).

In this group of secondary malabsorptive conditions, it will be seen that, apart from steatorrhoea and any deficiencies that may accrue from this, iron deficiency is common, vitamin B_{12} absorption is frequently disturbed, and sometimes vitamin B_{12} deficiency may follow prolonged iron deficiency; folic acid deficiency is not common.

There remains to be considered non-tropical sprue, idiopathic steatorrhoea or adult coeliac disease, as I prefer to call it. Adult coeliac disease is characterised by chronicity with relapse and remission, constitutional and familial features, certain variable vitamin deficiencies, variable haematological features and histologic changes in the jejunum which may be characteristic. Although in the past interest has been focused on the intestinal difficulties, it is now shifting to within the intestinal cell and to enzymatic defects in the body as a whole. Since the metabolism of the intestinal cell is the second highest in the body, it is not surprising that changes show up there. In the untreated patient with coeliac

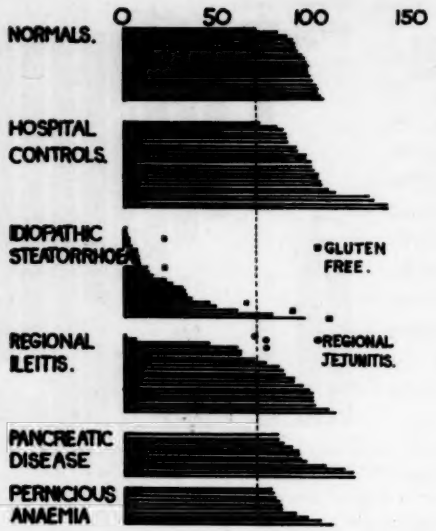

FIG. 5. Note the result of the folic acid excretion test. (Folic acid excretion index = Excretion after oral dose / Excretion after previous intramuscular injection × 100) The dotted line indicates lower limit of normality.

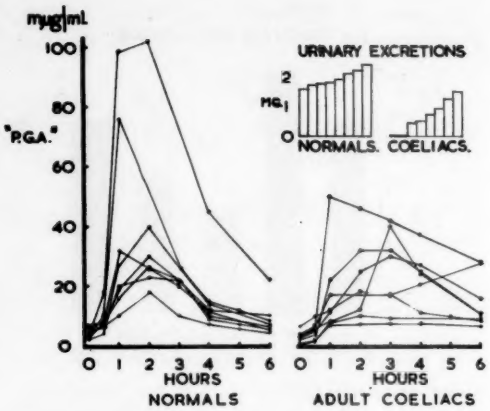

FIG. 6. Results of folic acid absorption test in eight patients with adult coeliac disease compared with those in six medical students and two hospital control subjects.

disease, there is a failure of iron absorption which is partially or completely corrected by adherence to a gluten-free diet or by the administration of steroids.[14] In patients with coeliac disease, iron deficiency anaemia is common but later in life macrocytic anaemias make their appearance and in about 40 per cent of adult patients there is interference with the absorption of vitamin B_{12}. In the majority of patients, this is not affected by the administration of intrinsic factor or antibiotics, but it is improved by the administration of steroids. It should be noted that in a few patients improvement does occur with the use of intrinsic factor.

Arising from our observations on the secondary malabsorptive conditions, two points should be considered. Is the tendency to chronic iron deficiency one of the factors responsible for the mucosal changes leading to the variable incidence of vitamin B_{12} deficiency in this disorder? My colleagues and I believe that this is a distinct possibility.[15] If so, the second question arises, can chronic iron deficiency cause sufficient cellular change to produce steatorrhoea? Again we believe that it is possible to separate a group of middle-aged and elderly women with hiatus hernia, long-standing menorrhagia or other source of chronic blood loss, in whom iron deficiency, vitamin B_{12} deficiency and steatorrhoea respond to iron therapy. In general, such patients appear to be well built and relatively well nourished.

We believe that iron and vitamin B_{12} deficiencies are essentially secondary features in adult coeliac disease. To us, folic acid would appear to be in a much more important and possibly key position. Girdwood[12] introduced a test in which the urinary excretion of folic acid following an intramuscular injection of folic acid is compared with that following an oral dose. We[13] have found that this test gives consistently abnormal results in adult coeliac disease (Figs. 5 and 6).

Girdwood[8] has encountered two patients with normal test results and stated that in 111 of 118 cases recorded, test results have been found to be abnormal. In our group one patient, a doctor in whom the results of the test were quite normal, proved to be sensitive to folic acid either whether administered orally or by injection. This patient, however, had taken the precaution to take antihistaminics at the time of the test. When the test was repeated without these drugs, the results were

abnormal. Three other patients with adult coeliac disease with similar sensitivity to folic acid are now under investigation. The results of the test, as applied to other conditions which may give rise to difficulties in diagnosis, are shown in Figure 5. The results seem clear-cut.

Girdwood implies that an abnormal test result signifies poor absorption of folic acid. We have preferred to call it an excretion test, believing that the evidence is insufficient as yet to attribute it to malabsorption in conditions other than those in which there is definite jejunal involvement, such as jejunitis. Although Spray and Witts[16] have published absorption curves showing poor blood levels following oral administration of folic acid, we have not been able to repeat these observations.

There is not a great deal of difference between absorption curves of the patients with adult coeliac disease and those of the normal control subjects: the lowest blood curve of the patients produced the highest excretion of folic acid in the urine. Chanarin, Anderson and Mollin[17] have found that when folic acid is administered intravenously it disappears much faster in adult coeliac disease than in other conditions. They have attributed this to folic acid deficiency in the tissues. We,[18] however, have called attention to the malutilisation of ascorbic acid that occurs in steatorrhoea which we were unable to attribute to poor absorption. Recently we[19] have been able to show that when ascorbic acid is administered intravenously to patients with adult coeliac disease it disappears more rapidly than in other conditions even when the plasma levels are normal and the patient's tissues are saturated with folic acid. The rapid disappearance of folic acid demonstrated by Chanarin may well be similar and may represent malutilisation of folic acid. This may also be related to the upset in tyrosine metabolism which we have demonstrated.[18]

It is of significance that test results have remained abnormal in patients on a gluten-free diet for periods up to four years even though there was clinical cure, no steatorrhoea and essentially normal haematologic findings. This is in contrast to the results reported in tropical sprue and in the few patients that we have been able to test. We therefore consider it possible that there is a fundamental defect in folic acid metabolism in patients with adult coeliac disease.

It has also become strikingly evident that gluten has a significant quantitative effect in adult coeliac disease. Children, however, can acquire tolerance for gluten, as Sheldon[20] has demonstrated, although the period of gluten free-diet necessary is at least eighteen to twenty-four months. The fraction of gluten concerned has been shown to be a polypeptide of unknown nature, and it is possible that certain other proteins may be implicated in rare cases. At present it does not seem possible to progress much further until the actual offending substance is isolated or synthesised.

It has been shown that a rise in blood glutamine occurs following the administration of gluten and, although this seems to be a somewhat inconstant feature, the question of the formation of a peptide-pteroylglutamate complex must be considered. That such a complex might act as a blocking agent in the folic acid cycle is suggested by the rapidity of haematologic response that may occasionally follow the administration of a gluten-free diet, a response comparable to that of a specific haematinic.[21] I have not considered other factors that might act as blocking agents, the clues to which may be found perhaps on chromatography of the urine.[19,22] I will conclude with a speculative hypothesis that adult coeliac disease is due to a constitutional enzymatic defect which is closely allied to folic acid metabolism and which is made evident by administration of gluten. In some patients this defect is so gross that it cannot be corrected even by a diet completely free of gluten.

More will be said later concerning tropical sprue. It does not appear to respond to a gluten-free diet and despite this does seem able to remit completely. I believe that it is a condition different from adult coeliac disease and much more closely related to jejunitis.

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The Malabsorption Syndrome in Man

Clinical and Pathologic Aspects

DAVID ADLERSBERG, M.D.*

THE clinical features of primary malabsorption syndrome (non-tropical sprue) to be presented in this summary are based on a study of more tnan 100 patients with this disorder observed at the Mount Sinai Hospital over a period of many years. In all the diagnosis was established by clinical and laboratory evidence; and all or most of the typical features of this syndrome appeared in all subjects at some time. Absence of gross gastrointestinal disease was established by roentgenologic and other laboratory and clinical procedures. Most of the patients had prolonged follow-up periods, averaging over five years.

There was a predominance of women in the present series, as previously reported from this Hospital.[1,2] Only Manson-Bahr noted a larger number of women with tropical sprue; others found the sex incidence approximately equal.

The average age of the patients at the time of first observation was forty-five years, range sixteen to seventy-three. When the patients were first seen the duration of symptoms ranged from one month to fifty-two years, with an average of 7.2 years. The long duration of symptoms is caused by the insidious onset, the vagueness of symptoms in the initial phases and the tendency to spontaneous remissions.

SIGNS AND SYMPTONS

The symptoms are summarized in order of frequency Figure 1. The most frequent complaints were the combination of diarrhea, weakness and weight loss but the leading symptom of diarrhea was absent in 4.2 per cent of the patients, while 1.2 per cent were constipated. These patients had considerable steatorrhea, nevertheless, and they may fall into the group of atypical or incomplete sprue. Loss of weight may be considerable: maximum 60 pounds, average 25.3 pounds. Glossitis and stomatitis, vague abdominal discomfort, bleeding, tetany, paresthesias and bone pains have been seen in the described order. Severe mental changes were seen in approximately 10 per cent of the patients. These ranged from frank psychosis to varying degrees of depression, anxiety and character disorders.

The pathologic signs observed are summarized in order of frequency in Figure 2. Emaciation was the leading sign. The initial body weight ranged from 67 to 167 pounds, mean weight 104 pounds. A large number of patients showed marked abdominal distention and dependent edema. Hepatomegaly and mild splenomegaly were seen in 31 and 6 per cent, respectively. Hypotension with systolic blood pressures below 100 mm. Hg was seen in approximately one-third of the patients. Less common manifestations were clubbing of the digits, pigmentation, dryness of the skin and abnormal neurologic signs.

CLINICAL PICTURE

In the typical case of non-tropical sprue, the patient is a middle-aged person with a long history of intermittent diarrhea, periods of marked weight loss, weakness, sore mouth and tongue, and abdominal complaints such as flatulence, mild pain or other discomfort. Often hemorrhagic manifestations, paresthesias or tetany may be present. The patient is pale, in poor nutritional status and may have clubbing of the digits; the abdomen is distended; the blood pressure is low.

From The Mount Sinai Hospital and Columbia University, New York, New York.

* Deceased.

Presented at the Symposium on Absorption Mechanisms and the Malabsorption Syndrome, under the sponsorship of The National Vitamin Foundation, Inc., March 3, 1959, New York, New York.

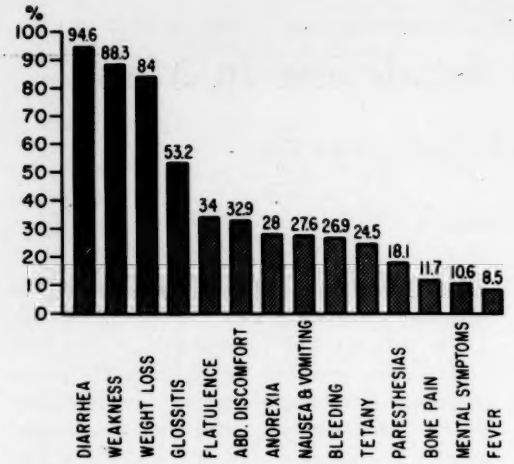


FIG. 1. Major symptoms observed in ninety-four patients with idiopathic sprue. (From: BOSSAK, E., WANG, C.-I. and ADLERSBERG, D. Clinical aspects of the malabsorption syndrome (idiopathic sprue): observations in 94 patients. *J. Mt. Sinai Hosp.*, 24: 286, 1957.)

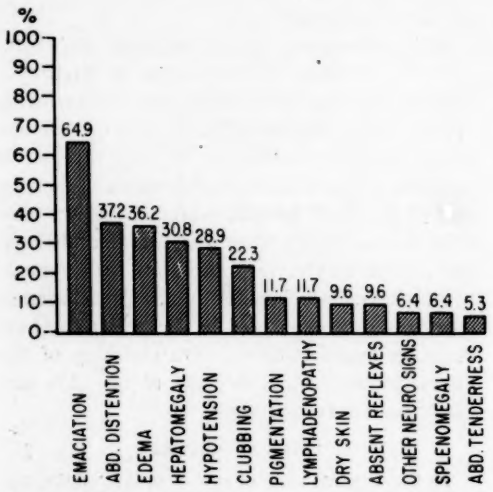


FIG 2. Major signs observed in same group of patients with idiopathic sprue as in Figure 1. (From: BOSSAK, E., WANG, C.-I. and ADLERSBERG, D. Clinical aspects of the malabsorption syndrome (idiopathic sprue): observations in 94 patients. *J. Mt. Sinai Hosp.*, 24: 286, 1957.)

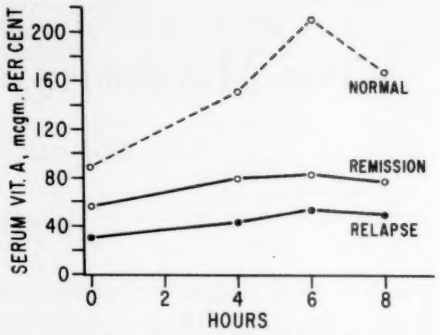


FIG. 3. The average vitamin A tolerance curve obtained in fifty-four patients with sprue in relapse compared with twenty-nine patients with sprue under clinical control. The normal absorption curve was the average of values obtained in ten healthy subjects. Note the progressive elevation of the serum vitamin A concentration after oral administration of the standard load of vitamin A with a gradual return toward normal levels; low fasting levels and flat curves in sprue in relapse and somewhat higher fasting levels but still flat character of the curve in sprue in remission. (From: ADLERSBERG, D., WANG, C.-I. and BOSSAK, E. Disturbances in protein and lipid metabolism in malabsorption syndrome. *J. Mt. Sinai Hosp.*, 24: 206, 1957.)

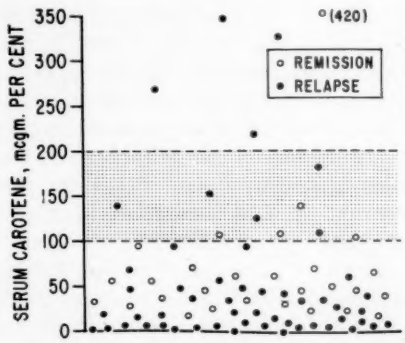
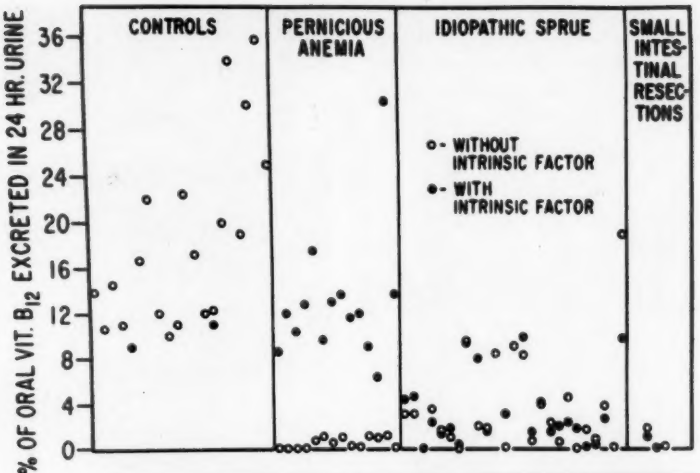


FIG. 4. Individual serum carotene levels in fifty-four patients with idiopathic sprue in relapse compared with twenty-eight patients with sprue under clinical control. The shaded area represents the range of normal serum carotene concentration. Note that the majority of patients with idiopathic sprue in relapse and in remission have serum carotene levels far below the normal range. (From: ADLERSBERG, D., WANG, C.-I., and BOSSAK, E. Disturbances in protein and lipid metabolism in malabsorption syndrome. *J. Mt. Sinai Hosp.*, 24: 206, 1957.)

Anemia is usually present and is often combined with a megaloblastic bone marrow in the presence of gastric acid. Serum albumin tends to be less than 3.5 gm./100 ml. and serum calcium less than 9 mg./100 ml. There is often an increase in alkaline phosphatase and a prolonged prothrombin time. All serum lipid



	CONTROLS	PERNICIOUS ANEMIA		IDIOPATHIC SPRUE		SMALL INTESTINAL RESECTIONS	
	NO I.F. (o)	NO I.F. (o)	I.F. (•)	NO I.F. (o)	I.F. (•)	NO I.F. (o)	I.F. (•)
AVERAGE	17.9	0.5	12.9	3.6	3.3	0.63	0.6
RANGE	9-36	0-1.2	6.3-30.5	0-19	0-10	0-1.8	0-1.1

FIG. 5. Scattergraph of urinary excretion tests in control subjects as well as in patients with pernicious anemia, idiopathic sprue and extensive intestinal resections. The open circles represent the excretion of labeled vitamin B_{12} given orally without intrinsic factor. The closed circles represent the excretion of labeled vitamin B_{12} given orally with intrinsic factor. Each test on the same patient is placed in vertical alignment. Note that the abnormally low excretion of vitamin B_{12} in patients with pernicious anemia can be reverted to normal by the addition of intrinsic factor (I.F.) whereas in patients with idiopathic sprue the excretion levels remain low with or without the addition of intrinsic factor. (From: OXENHORN, S., ESTREN, S., AND ADLERSBERG, D. *J. Mt. Sinai Hosp.*, 24: 232, 1957.[3])

fractions are low, along with low levels of serum vitamin A and carotene. Steatorrhea is present. On roentgenologic examination the bones are demineralized (osteomalacia and/or osteoporosis). There are evidences of diminished glucose and xylose absorption, of impaired fat, vitamin A and vitamin B_{12} absorption (Figs. 3, 4 and 5).[2,3] Other problems, such as the absorption of folic acid, iron, protein, water and electrolytes will not be discussed here.

Roentgenologic studies of the small bowel are mandatory in every patient with primary malabsorption syndrome. Organic changes of the gastrointestinal tract must be ruled out before the diagnosis of primary malabsorption syndrome is established. In the latter, characteristic changes of the pattern of the small bowel can be seen which consist of pronounced dilatation of small bowel loops, segmentation

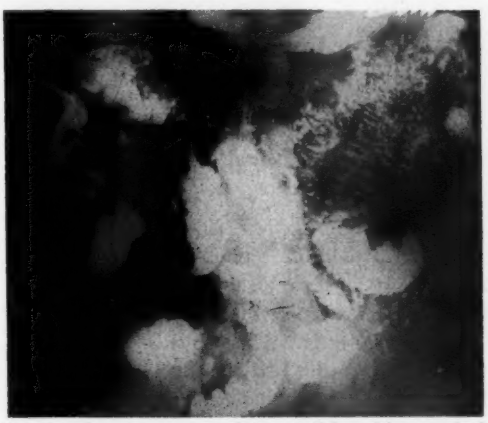


FIG. 6. Roentgenogram of the small bowel in a typical case of non-tropical sprue. The "sprue pattern" is characterized by considerable dilatation of the loops segmentation and evidences of hypersecretion. (From: ADLERSBERG, D., MARSHAK, R. H., COLCHER, H., DRACHMAN, S., FRIEDMAN, A. I. and WANG, C.-I. *Gastroenterology*, 26: 548, 1954.[4])

TABLE I

Comparison of Clinical and Laboratory Data in Puerto Rican and Non-Puerto Rican Patients with Sprue

Data	Non-Puerto Rican	Puerto Rican
Patients (no.)	71	23
Duration of symptoms (yr.)	8.8	2.3
Glossitis (%)	46.5	74.0
Bleeding (%)	33.8	8.7
Tetany (%)	29.6	8.7
Clubbing (%)	26.8	8.7
Hepatomegaly (%)	25.4	52.2
Paresthesias (%)	15.5	26.1
Splenomegaly (%)	2.8	17.4
Megaloblastic bone marrow (%)	9.5	89.0
Hemoglobin <5 gm. % (%)	7.0	34.8
Leukopenia (%)	24.6	74.0
Bone demineralization (%)	65.6	20.0
Calcium <7 mg. % (%)	38.9	5.9
Mortality (%)	26.7	4.5
Cured (%)	1.7	21.7

* From: Bossak, E., Wang, C.-I. and Adlersberg, D. Clinical aspects of the malabsorption syndrome (idiopathic sprue): observations in 94 patients. *J. Mt. Sinai Hosp.*, 24: 286, 1957.

resulting in "puddling" of the barium and evidence of excessive amounts of secretion in the small intestinal tract (Fig. 6). Approximately 90 per cent of the patients with primary malabsorption syndrome present the "sprue pattern" of the small bowel on roentgenographic examination.[4] Thus, this finding may represent an important diagnostic criterion.

Patients with milder or incomplete cases may present diagnostic difficulties. Steatorrhea without diarrhea as well as severe bleeding (hypoprothrombinemia), fractures (osteomalacia) and intractable edema (hypoproteinemia) may mask the basic disorder.

Puerto Rican patients with sprue (seen in New York) differ in several respects from non-Puerto Ricans with sprue. They have a higher incidence of glossitis and hepatomegaly and a lower incidence of bleeding, tetany and clubbing; a higher incidence of severe anemia, macrocytosis and leukopenia; higher levels of serum calcium and a lower incidence of bone demineralization. Since the majority of these patients had symptoms before emigrating to New York, they may be presenting the tropical variety of sprue (Table I).[5]

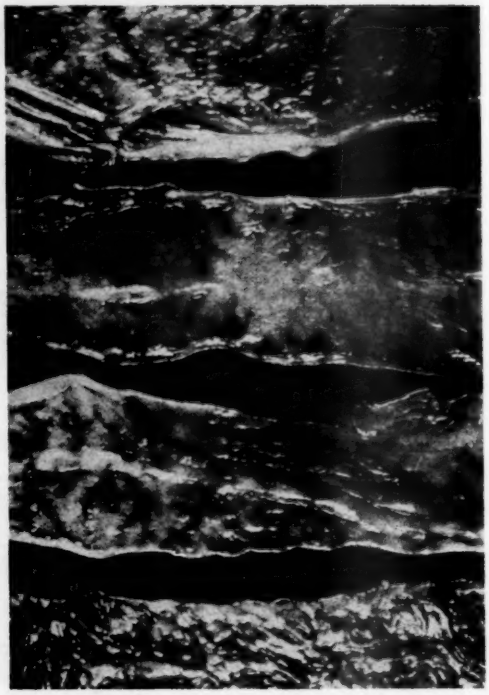


Fig. 7. Atrophy and thinning of wall of small bowel in a patient with nontropical sprue. (From: Himes, H. W. and Adlersberg, D. Pathologic studies in idiopathic sprue. *J. Mt. Sinai Hosp.*, 24: 251, 1957.)

HISTOLOGIC ASPECTS

Clinical evidence would suggest that the primary malabsorption syndrome is caused by a derangement or a defect in the small bowel. Since a number of patients with primary malabsorption syndrome present some morphologic change in the small bowel at autopsy and biopsy, it would appear that these changes are causally related to the clinical manifestations.[6] The pathologic changes in the small bowel seen in advanced cases are characterized macroscopically and microscopically by marked atrophy of the small intestinal mucosa (Fig. 7). In some instances, blunting and thickening of the villi, in others, almost complete atrophy of the villous layer, may be seen (Figs. 8 and 9). These changes obviously lessen the surface area available for absorption. The question of

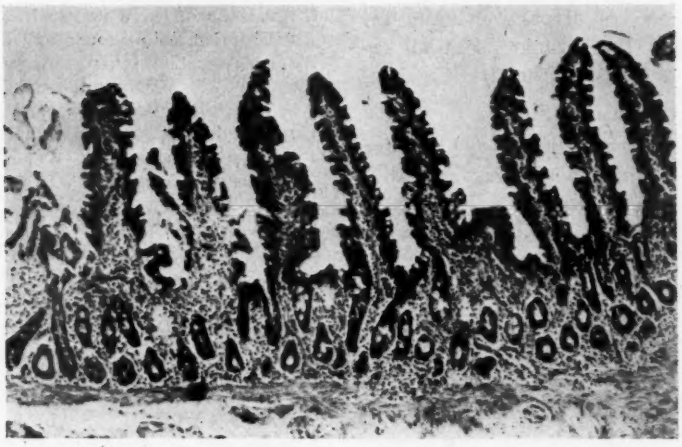


FIG. 8. Jejunal biopsy specimen from a normal control subject. Note finger-like appearance of normal villi. (From: HIMES, H. W. and ADLERSBERG, D. *Gastroenterology*, 35: 142, 1958.[6])

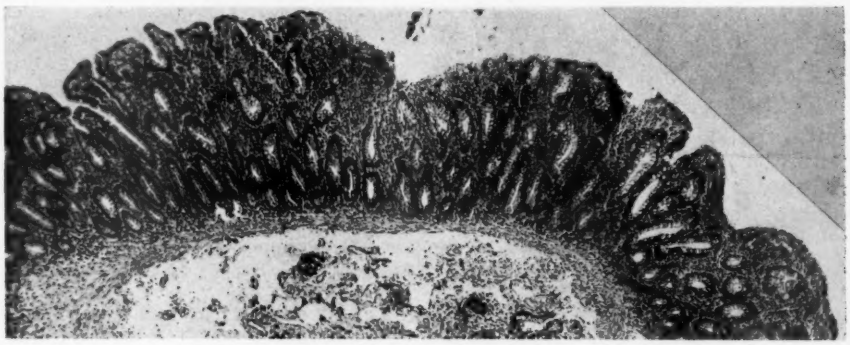


FIG. 9. Jejunal biopsy by the oral route in a patient with non-tropical sprue of long duration. Note atrophy of the mucosa with complete distortion of the villous layer.

the reversibility of these structural changes in sprue is still open. Interesting information concerning the ultimate structure of the jejunal mucosa is being obtained presently by electron microscopic studies.

The changes in the small bowel may be the result of alterations in small bowel motility, absorption of abnormal products or lack of specific nutritional factors such as vitamin B_{12}, folic acid and other as yet unidentified compounds. They may be the result of some deranged enzymatic mechanisms in the intestinal mucosa and thus be a part of the basic disturbance in malabsorption. Experience with small intestinal biopsies seems to indicate that the severe atrophy of the mucosa is, to a certain extent, characteristic of non-tropical sprue. In tropical sprue, the changes observed by others and us seem to be milder in character.

Patients with pernicious anemia, prolonged steatorrhea caused by pancreatic disease and with chronic diarrhea of various causes do not show the atrophy of the small intestinal mucosa seen in the primary malabsorption syndrome. The same degree of atrophy of the mucosa may be seen in patients with non-tropical sprue in full remission, normal nutritional status and in absence of steatorrhea. The question arises then, as to how normal absorption can be maintained during full remission despite severe atrophy of the mucosa. Whether atrophy of the small bowel mucosa

and malabsorption are actually causally related and whether the atrophy is at all reversible remain to be established.[7]

SUMMARY

Primary malabsorption syndrome includes several clinical entities which are probably varieties of the same inborn metabolic disorder. It is not clear at present whether the characteristic changes of atrophy of the small intestinal mucosa seen at autopsy and in biopsy specimen of patients with the primary malabsorption syndrome are due to a specific enzymatic or nutritional defect or whether they result from some toxic or other influences.

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Hematologic Aspects of Sprue

FRANK H. GARDNER, M.D.*

THE FREQUENCY of anemia in the sprue syndrome has stimulated clinical investigation in the evaluation of hematinics for its therapy. In some respects, sprue has been considered a hematologic disorder. The resultant immediate treatment of the anemia has in many instances allowed the patient to resume activity and employment.

In the Caribbean area, the presence of macrocytic anemia with megaloblastic bone marrow has been necessary to authenticate a diagnosis of sprue.[1] Such criteria, however, have caused confusion in the comparison of problems of malabsorption in various other areas of the world. British workers in India, dealing primarily with military personnel, have not observed a large incidence of megaloblastic anemia.[2] This difference has resulted in speculation that the sprue syndrome observed in the Caribbean area is actually an advanced form of nutritional anemia. Although two types of sprue may be inferred from the presence or type of anemia, in reality such differences reflect: (1) the duration of the disorder, and (2) the predisposition for onset of anemia because of previous nutritional deficiencies. The proposed classification of Stefanini[3] can be used with profit to separate the hematologic complications (Table I).

CLASSIFICATION

Stage I (Early Phase): The early manifestations of sprue are seen only in tropical areas. Diarrhea, asthenia and loss of weight may be present for weeks or months before diagnosis is made. Most generally, these patients have been found among military personnel. The malabsorption syndrome observed among such personnel in Puerto Rico would be classified in stage I.[4] The disorder is often self-limited with recovery not related to specific therapy. These early cases have emphasized that malabsorption in the small bowel may be present for months without hematologic complications.

Stage II (Deficiency Phase): In areas with widespread nutritional deficits, malabsorption for short intervals (months) rapidly accentuates vitamin deficiencies.[5] Iron deficiency from poor diet or food habits may accentuate hypochromic rather than macrocytic anemias. Macrocytosis of the red cells may be associated with early erythroblast alterations of the bone marrow as absorption of folic acid and vitamin B_{12} are impaired. If serum hypoferremia emphasizes intermediate forms of megaloblasts, then the "classic" megaloblastic marrow is not observed.[6] Bowel function returns to normal after therapy with folic acid or vitamin B_{12}. Occasionally oral antibiotics have been successful in achieving clinical remissions,[7,8] but this response is confined to tropical areas. The anemia itself responds to folic acid and to oral iron medications.

Stage III (Macrocytic Anemia): Prolonged depletion of body stores following malabsorption results in megaloblastic changes in bone marrow. Jejunal biopsies have demonstrated alterations in the intestinal villi in both tropical and non-tropical sprue. While administration of folic acid or vitamin B_{12} will cause a hematologic remission, the abnormal villi in the small intestine persist. Malabsorption of all foodstuffs persists in varying degrees and a hematologic relapse may be anticipated unless

From the Richard C. Curtis Hematology Laboratory, Peter Bent Brigham Hospital, Boston, Massachusetts.

* Senior Associate in Medicine, Peter Bent Brigham Hospital and Assistant Professor of Medicine, Harvard Medical School, Boston, Massachusetts.

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TABLE I
Clinical Phases of Sprue

Classification	Duration	Hematologic Features	Bone Marrow	Bowel Malabsorption	X-ray Pattern of Small Bowel	Symptoms
Stage I (early phase): tropical sprue	Weeks to months	Normal	Normal	None to mild	Early changes	Mild
Stage II (deficiency phase): tropical sprue, ? early celiac disease	Months	Hypochromia and/or macrocytosis	± early erythroblast changes	Moderate to severe	Abnormal	Secondary deficiencies
Stage III (macrocytic anemia): tropical sprue or non-tropical disease	Months to years	Hypochromia and/or macrocytosis	+ megaloblast changes	Severe	Abnormal	Multiple deficiencies

supplemental oral therapy is administered.

In the majority of adult patients with idiopathic steatorrhea (non-tropical sprue) studied in the temperature zone macrocytic anemia will develop if vitamin therapy is withheld. Patients with a gluten enteropathy may be classified in stage III. Exclusion of gluten from the diet will allow adequate absorption of hematinics to correct hematologic abnormalities.[9]

The adult patient with malabsorption is never seen in the early stages of the disease, since the disorder of the bowel has persisted for many years before diagnosis is usually considered.[10] Estren[11] observed macrocytosis in 58 per cent of a series of patients with sprue studied in New York. Twenty-three of the ninty-four patients were Puerto Ricans and of these 89 per cent had a megaloblastic bone marrow in contrast to 9.5 per cent of the other seventy-one patients. Personal experience would intimate that this group exhibited a greater frequency of macrocytosis than is usually observed in the temperature zone of the United States. Studies by Cooke et al.[12] and Innes[13] in Great Britian indicated macrocytosis of the red cells was present in two-thirds of their patients with non-tropical sprue. Patients with sprue studied in our university teaching hospitals are evaluated only after long periods of illness. The widespread prescription and self-medication with multivitamin preparations alter the hematologic pattern. Macrocytosis of the red cells would probably be present to the same extent if such vitamin therapy were not available to the patient for self-medication.

The macrocytic red cells associated with megaloblastic bone marrow changes in tropical sprue have a shortened life span *in vivo* similar to the macrocytes observed in untreated pernicious anemia[14] (Fig. 1). Although macrocytosis and hypochromia of the red cells persist during therapy with folic acid, the life span of the red cells does return to the normal range.

SPECIFIC HEMATINICS IN THE SPRUE SYNDROME

Vitamin B_{12}: Patients with tropical sprue have decreased serum vitamin B_{12} levels only if malabsorption has been of sufficient duration to deplete body stores (stage III).[5] The patient without anemia (early phase) has serum levels within normal limits.[4] If the megaloblastic anemia of tropical sprue is treated with folic acid, a hematologic remission is obtained. However, the serum vitamin B_{12} levels may remain in the low pretreatment range, often below 100 $\mu\mu$g./ml. (Euglena gracilis assay) which is the range observed in the untreated patient with pernicious anemia. These depressed serum levels may be present for months or years without development of neurologic complications or hematologic relapses.[5] Certainly, the patients with tropical sprue observed in Puerto Rico had an adequate diet of vitamin B_{12} if bowel absorption was sufficient. The untreated patient with tropical sprue has im-

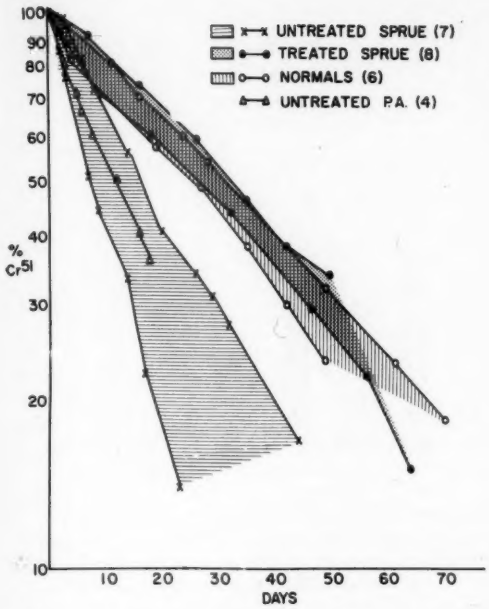

FIG. 1. Life span of red cells in tropical sprue labeled with radioactive sodium chromate. The outline of each shaded area represents the range of values observed in each group. The number of subjects studied is listed in parentheses. A mean curve for four patients with megaloblastic untreated pernicious anemia (P. A.) is drawn to compare with the life span observed in the megaloblastic anemia of untreated tropical sprue. The patients treated with folic acid have a normal life span despite continued macrocytosis of the red cells.

paired absorption of Co^{60}-labeled vitamin B_{12}, but the impairment is not as marked as the serum levels would suggest. These patients in hematologic remission during folic acid therapy had a greater fecal excretion of the labeled vitamin B_{12} than noted in the untreated patients with sprue, probably emphasizing that malabsorption had been present for a longer interval with more irreversible damage to the bowel mucosa.[5] Following antibiotic therapy, Co^{60}-vitamin B_{12} absorption was improved in some cases.[15]

The patient with non-tropical sprue has a more profound pathologic lesion than the patient with tropical sprue, for in most instances he has had an altered bowel mucosa for years. The levels of serum vitamin B_{12} are low in many such patients studied.[16,17] Absorption of vitamin B_{12} as measured by fecal or urinary excretion of radioactive labeled cobalt is depressed.[18] Some improvement is noted after the initiation of a gluten-free diet. The larger series of Oxenhorn et al.[19] has not revealed enhanced oral absorption of vitamin B_{12} by the addition of 30 mg. of purified intrinsic factor. However, with larger doses of intrinsic factor (200 to 1,000 mg.), some enhancement of radioactive vitamin B_{12} has been observed in patients with sprue.[20] Since the amount of intrinsic factor is far in excess of the dosage required in pernicious anemia, some consideration of this phenomenon is warranted. Two aspects of effective vitamin B_{12} absorption will be discussed; namely, the morphologic changes in surface area of the bowel and the effects of the intestinal bacterial flora.

Butterworth and Perez-Santiago[21] have proposed that the abnormal villi in sprue reduce the intraluminal surface of the small bowel area to about 25 per cent of normal The microvilli in each columnar cell increase the absorptive area twentyfold.[22] In tropical and non-tropical sprue the microvilli are fused, blunted and decreased in number. Loss of one-half of the microvilli would then reduce the surface area to 12 per cent of normal. Inadequate surface area may be an acceptable explanation for the impaired absorption of vitamin B_{12} or other nutrients. Fecal excretion of vitamin B_{12} represents only one-third of the biliary vitamin B_{12} loss which indicates conservation of the vitamin by the enterohepatic circulation.[23] Changes in the mucosa and decreased surface area may not allow efficient reutilization by this pathway. Finally, intravenous infusions of I^{131}-polyvinylprolidone (P.V.P.) with a molecular weight of 40,000, have demonstrated an increased secretion (oozing) of the material into the bowel lumen of patients with sprue.[24] One may speculate whether or not vitamin B_{12} bound to serum proteins could be excreted in excessive amounts with impaired reabsorption.

Recent clinical and laboratory observations have demonstrated that vitamin B_{12} is absorbed for the most part in the ileum.[25] Hence, it is absorbed in the area of the small bowel most likely to have a "resident" bacterial flora and the first portion of small bowel to be invaded by

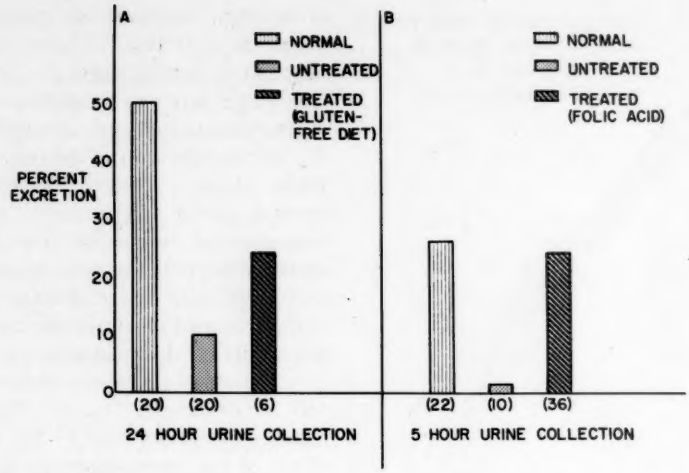

FIG. 2. Oral absorption of folic acid. Patients with non-tropical sprue have an incomplete recovery following therapy with a gluten-free diet. Patients with tropical sprue have normal absorption during therapy with folic acid. The bar graphs rerpesent the mean value of each group. The number of each group is listed in parentheses. Non-tropical sprue (A) data derived from Cox et al.[33] and tropical sprue (B) from Butterworth et al.[31]

a cephalad overgrowth of a luxuriant colon flora. The clinical improvement associated with antibiotic therapy in tropical sprue[7,8] may well reflect sterilization of bacterial flora in the ileum of the ascending colon. Unfortunately, there is no information regarding bacterial flora in the ileum in malabsorption syndromes, but current reports have emphasized that there is no abnormal bacterial growth in the jejunum.[26,27] Hence, in the area of optimal vitamin B_{12} absorption, competition for this substance by bacterial binding may decrease by means of antibiotic therapy. This hypothesis has been especially well substantiated in the "blind loop" syndrome of the ileum after surgical procedures.[28] *In vitro* studies of E. Coli organisms obtained during surgical correction of blind loops demonstrated that the bacteria could "bind" radioactive vitamin B_{12} from the culture media.[29] This suggests that bacterial flora could deprive the patient with blind loops of the small bowel of vitamin B_{12}. Clinical improvement during a gluten-free diet program may be associated with a decrease in the number of digested nutrients passing into the ileum and colon able to support a luxuriant bacterial flora. As the bacterial competition decreases, more vitamin B_{12} is available for absorption in the ileum. This absorption may be enhanced by administration of large amounts of intrinsic factor.[30]

Folic Acid: There is no information available regarding the absorption of folic acid in the early phases of malabsorption (stages I and II). Following the onset of megaloblastic anemia in tropical sprue (stage III), there is marked impairment of absorption after a 5 mg. oral dose of folic acid.[31] Non-tropical sprue has a similar defect in absorption.[32,33] With correction of the megaloblastic anemia by folic acid therapy, the patient with tropical sprue can absorb folic acid with the same efficiency as normal subjects. Antibiotic therapy will also improve folic acid absorption in tropical sprue,[34] suggesting the role of bacterial competition intraluminally as noted above with vitamin B_{12}. Institution of a gluten-free diet improves folic acid absorption in non-tropical sprue to about half the normal value[33] (Fig. 2). The irreversible damage to the bowel probably precludes improvement to the normal range for such patients.

The untreated patient with tropical sprue has a leukocyte concentration of folic acid at the

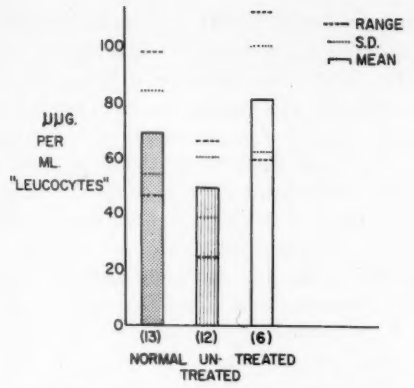

FIG. 3. White blood cell folic acid values in tropical sprue. The untreated patient with tropical sprue has only minimal depletion of tissue stores of folic acid as measured by white cell concentrations. Treatment with oral folic acid elevates the "buffy coat" values to normal range. The number of subjects studied is listed in parentheses. S. D. represents one standard deviation. Data derived from Butterworth et al.[31]

lower limits of normal values which implies only minimal tissue depletion of the vitamin[31] (Fig. 3). These observations suggest that the megaloblastic anemia cannot be attributed to a nutritional deficiency of folic acid. Also, the untreated patient with tropical sprue is capable of converting glycine to serine, a reaction requiring folic acid.[35] Hence, some defect in folic acid derivatives or in enzymes acting as cofactors appears to be the primary factor in the disease.

Butterworth et al. have demonstrated that ingestion of large amounts of DL-serine will initiate a reticulocyte response in the untreated patient with tropical sprue and megaloblastic anemia. These workers have proposed that the use of serine would spare folic acid in the conversion of glycine to serine.[36] Such sparing action would then allow greater formation of citrovorum factor (folinic acid), thought to be ten times as potent as folic acid in the treatment of sprue. These same workers have demonstrated an increased urinary excretion of formiminoglutamic acid (FIGLU), a metabolite which has been found in the urine of animals with folic acid deficiency. In the presence of tetrahydrofolic acid FIGLU participates in the formation of citrovorum factor (folinic acid). The patient with tropical sprue seemingly cannot transfer the formimino group which is present in excess. He also has a defective transfer enzyme, FIGLU transferase. The pharmacologic dosages of folic acid used in therapy may "drive" the formation of adequate folinic acid by mass action rather than replenishing a nutritional deficiency.

In contrast, patients with non-tropical sprue (gluten enteropathy) do not completely regain the ability to absorb folic acid. This may be related to a more severe morphologic lesion of the mucosa, or an enzyme defect in the mucosal cells.[17] Folic acid is absorbed in the proximal portion of the small bowel (in contrast to the absorption of vitamin B_{12} in the distal portion).

Iron: Absorption of iron is affected early in the onset of malabsorption in tropical sprue.[4] Patients without hypoferremia and anemia have impaired absorption, and this early defect may explain the frequency of iron deficiency anemias noted in the sprue syndromes. Patients with megaloblastic bone marrow changes usually have hypoferremia. For optimal hematologic recovery oral iron supplements should be administered along with folic acid. Although absorption studies have been performed by measuring serum iron levels after oral doses of iron salts, the hypoferremia excludes any adequate interpretation. The serum levels indicate a "flat" tolerance test indicating poor absorption of iron, but the rapid plasma clearance of the absorbed iron associated with iron deficiency excludes any adequate interpretation. The fecal excretion studies with radioactive iron salts carried out by Badenoch and Callender[37] in non-tropical sprue give a more accurate interpretation and show a persistent malabsorption of iron. The hematologic improvement and elevated serum iron levels following the use of a gluten-free diet imply that absorption of iron will improve in non-tropical sprue.

In rats, chronic iron deficiency is associated with impaired formation of intrinsic factor and decreased absorption of vitamin B_{12}. These alterations were not related to morphologic changes in the small bowel, but some animals had atrophy of the gastric mucosa.[38] Cooke[39] has emphasized the value of iron salts to im-

TABLE II

Proposed Mechanisms That May Interfere with Utilization of Hematinics in Sprue

Vitamin B_{12}
Histologic defect of small bowel mucosa with malabsorption
Bacterial competition (ileum)
Nutrition and non-specific binding of vitamin B_{12}
Non-specific binding or utilization of intrinsic factor
Folic Acid
Histologic defect of small bowel mucosa with malabsorption
Intraluminal bacterial competition
Enzyme defect of FIGLU transferase
Inadequate ascorbic acid available for formation of folinic acid
Iron
Histologic defect of small bowel mucosa with malabsorption
Binding of iron by abnormal mucoproteins and foodstuffs in bowel lumen from delayed motility
Ascorbic Acid
Histologic defect of small bowel mucosa with malabsorption
Intraluminal bacterial competition to support metabolism of aromatic amino acids.
Inadequate serum vitamin B_{12} concentrations to maintain ascorbic acid in reduced form
Increased demands for tissue repair of abnormal mucosa

prove serum vitamin B_{12} levels in some patients with the non-tropical sprue syndrome. The subsequently improved metabolism of vitamin B_{12} in these patients emphasizes the importance of the administration of oral iron salts in malabsorption syndromes for complete hematologic improvement.

Ascorbic Acid: Since many simple molecular substances are poorly absorbed in the sprue syndrome,[5] one may assume that the altered mucosa impairs the absorption of ascorbic acid. Plasma levels are low in non-tropical sprue.[40] Large doses of ascorbic acid are necessary to elevate plasma levels to the normal range. These low plasma concentrations appear to be related to levels of serum vitamin B_{12}. After vitamin B_{12} therapy, plasma ascorbic acid concentration was closer to the normal range, suggesting that vitamin B_{12} may be necessary to prevent oxidation of ascorbic acid.[40]

The major source of urinary phenolic compounds is derived from bacterial action on tyrosine and phenylalanine. Ascorbic acid is necessary for the complete degradation of these aromatic amino acids by tryrosinase. The intermediate metabolites (*p*-hydroxyphenylacetic acid, *p*-hydroxyphenylpyruvic aicd, *p*-cresol) are excreted in excess concentrations in the patient with scurvy on a high protein diet.[41] These derivatives disappear with adequate administration of vitamin C. Boscott and Cooke[42] noted an increased urinary excretion of *p*-hydroxyphenylacetic acid in five patients with non-tropical sprue. Massive doses of ascorbic acid eliminated this metabolite from the urine with an associated elevation of peripheral blood values. Several possible explanations can be attributed to these observations: (1) an overgrown bacterial flora had increased demands for ascorbic acid for proteolytic metabolism; (2) the inflammatory changes in the mucosa are associated with increased proliferation of epithelium requiring ascorbic acid for "tissue repair;" and (3) inadequate ascorbic acid is available for conversion of folic acid to folinic acid to participate in erythropoiesis. These explanations should be evaluated in the patient with tropical sprue and megaloblastic anemia. The relationship of vitamin C and the bacterial flora should be studied during antibiotic therapy.

SUMMARY

Knowledge of the duration of malabsorption is helpful in comparing the hematologic defects seen in various types and phases of the sprue syndromes. Adequate therapies are available to correct the anemias observed in these disorders. A summary listing of the malutilization of the accepted hematinics is presented in Table II. The use of microbiologic assay and labeled nutrients has allowed a better understanding of erythropoiesis. Evidence is accumulating to imply that the factors initiating anemia are not nutritional depletion of hematinics *per se* but rather involvement of enzymes or constitutional defects.

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Management of Primary Intestinal Malabsorption

A. B. FRENCH, M.D.,* H. MARVIN POLLARD, M.D.,† M. ISHIKAWA, M.D.,‡
J. T. RATNER, M.D.§ AND M. M. ABOWD, B.S.‖

THE OUTLOOK for the patient with primary intestinal malabsorption has changed dramatically in the past decade. Ten years ago treatment of tropical sprue with liver extract produced reasonably good results but treatment of celiac disease or non-tropical sprue with the same agent was of questionable benefit. Dietary management seemed beneficial in some instances but there was little agreement about which type of diet helped.

In 1946, folic acid[1–11] was introduced into the therapy of tropical sprue. While the use of folic acid does not dramatically improve the previous good results of treatment with liver extract, it has led to better understanding of the fact that folic acid deficiency is consistently present in tropical sprue and that symptoms disappear or are remarkably improved when the deficiency is corrected.

In 1950, Dicke[12] reported that patients with celiac disease benefited from wheat restriction and Dicke, Weyers and van de Kamer[13] then showed that the presence of wheat, rye or oat protein in the diet of these patients is harmful and that its withdrawal consistently leads to decrease in steatorrhea. The harmful effect of these grain proteins, which as a group are called glutens, was found to be concentrated in the gliadin fraction in wheat and to correlate in a roughly quantitative way with the proportion of glutamine incorporated in the protein. In 1952[14] it was reported that the beneficial effect of a gluten-free diet in celiac disease applied also to non-tropical sprue, so that while it is far from clear that either celiac disease or non-tropical sprue is due to sensitivity to gluten, a tool is now available which brings about dramatic improvement in these patients, and which may help us to better understand both normal and abnormal intestinal absorption.

At about the same time that the harmful effect of gluten was first reported, other groups began to report the beneficial effect of treatment with ACTH or cortisone in non-tropical sprue[15–20] as well as in certain types of secondary sprue.[21–22] Antibiotic therapy has also been found useful.[24,25] Thus, dramatic therapeutic technics have become available to make management of primary malabsorption as rewarding today as it had been frustrating in the past.

The present report summarizes these recent advances and presents the technics and results of management as applied in one hospital.

DEFINITION OF TERMS

It is first necessary to define the terminology of the diseases whose management is under dis-

From the Gastrointestinal Section of the Department of Internal Medicine, The University of Michigan Medical School, Ann Arbor, Michigan.

* Associate Professor of Internal Medicine; † Professor of Internal Medicine, The University of Michigan Medical School. ‡ Present address: Tohoku University Medical School, Sendai, Japan. § Instructor in Internal Medicine, The University of Michigan Medical School. ‖ Present address: Fostoria, Ohio.

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cussion. We use the term primary malabsorption to designate the three diseases: tropical sprue, non-tropical sprue and celiac disease. While there is some logic in considering these three as variants of a single disease, evaluation of therapy requires that each be considered separately. "Non-tropical sprue" is considered synonymous with "idiopathic steatorrhea." For purposes of this review "adult gluten-induced enteropathy" and "adult celiac disease" are also considered synonymous with non-tropical sprue, although they may actually represent instead major and overlapping subgroups. Tropical sprue has at least two variants, a milder and relatively acute form often seen in military personnel or other transients, and a more severe and chronic form commonly seen in native populations in the Caribbean or Far East. It is probable that these represent different stages of the same disease, modified by the pre-existing nutritional state, but the differences between them must be considered in evaluation of therapy.

LIVER AND FOLIC ACID

The use of liver soup in tropical sprue, recommended by Sir Patrick Manson as early as 1883, was one of the clues that led Minot and Murphy[26,27] to treat pernicious anemia with large amounts of liver. The administration of liver[28–31] or liver extract by mouth[32,33] to patients with tropical sprue was helpful but not as consistently so as in pernicious anemia. However, with the use of parenteral liver extract[34–37] it was possible to produce a consistent hematologic response and usually a striking decrease in symptoms. When groups working with Spies,[6–10] Moore,[4] Darby,[1–3] Manson-Bahr[5] and others[11] reported that folic acid administered either parenterally or orally produced the same response as did parenteral liver extract, it became evident that folic acid deficiency is responsible for a major part of the clinical picture of tropical sprue. It appears probable that the therapeutic effects of liver or marmite (yeast) administration were mainly due to their folic acid and vitamin B_{12} content.

Many of these optimistic reports concerned themselves chiefly with the anemia and glossitis of tropical sprue. It was stated that in most patients diarrhea, distention and flatulence decreased and the patients gained weight, but these results were not as consistently emphasized as the hematologic response. In the epidemics of tropical sprue in military personnel in India during the war, it was commonly found that liver therapy was followed by a good initial response but that gastrointestinal symptoms did not completely disappear.[38,39] Use of oral rather than parenteral adminstration of liver extract in most of these patients may have led to suboptimal response. Follow-up studies of military patients with tropical sprue treated with liver or folic acid found little residual disability.[40,41] However, tropical sprue in military personnel in India or in Puerto Rico seems milder and less chronic than the tropical sprue found in the native populations.[42,43] In a ten-year follow-up study of the Puerto Rican patients with tropical sprue originally treated by Castle and associates[35], Rodriguez-Molina[44] found that although glossitis, diarrhea, distention, abdominal distress and anemia had improved in all, some residual symptoms were present in most patients in spite of continuing liver therapy.

The effect of liver and folic acid therapy on intestinal absorption has been studied much less carefully than the effect on formation of red cells and some points remain obscure. Fairley[45] found a decrease in the proportion of fat in dried stool from an average of 48 per cent to an average of 26 per cent when patients with tropical sprue were treated with liver extract. Unfortunately, subsequent studies have shown that it is not acceptable to express fecal fat in this way since non-fat dry bulk of stool changes as well as fecal fat.[46] Fairley[45] also noted improvement in the flat glucose tolerance curves of patients with tropical sprue following liver therapy. This has also been noted after liver therapy in patients with celiac disease[47] and in patients with tropical sprue after folic acid therapy.[3,42] Barker and Rhoads[48] found that blood lipid curves measured by the Van Slyke lipid carbon method were flat in untreated non-tropical sprue and returned to normal after treatment with parenteral liver extract. Gardner noticed similar improvement in vitamin A and serum turbidity (lipemia) curves in mili-

tary patients with mild to moderate tropical sprue treated with folic acid.[42] However, folic acid therapy produced little change in these indirect indices of fat absorption in Gardner's Puerto Rican natives with more severe tropical sprue[43] or in the patients with non-tropical sprue studied by Darby et al.[3]

In the first metabolic balance studies carried out in patients with primary malabsorption, Bassett et al.[49] found no improvement in fecal fat, nitrogen, calcium and phosphorus losses of three patients with non-tropical sprue treated for a month or more with daily intramuscular injections of liver extract and studied throughout the period of treatment. Moore and co-workers,[4] Darby et al.[3] and, in more detailed studies, Black, Bound and Fourman,[50] Fourman et al.[51] and Woodruff[52] showed that, although a hematologic and symptomatic response consistently occurred to folic acid or liver therapy in patients with non-tropical sprue, fecal fat showed little change. Black, Bound and Fourman[50] noted improvement in steatorrhea of a patient with tropical sprue on yeast extract therapy but the difference may have been due to larger dosage or natural variation in one patient. Davidson, Girdwood and Innes[53] found neither improvement in steatorrhea nor a consistent hematologic response to treatment with folic acid in patients with tropical sprue. Although this failure to obtain a hematologic response to folic acid is uncommon in tropical sprue, Weir and Comfort[54] and Badenoch[55] observed neither hematologic, symptomatic or absorptive response to administration of folic acid in non-tropical sprue. In celiac disease, although single instances of hematologic response are reported, particularly in the presence of macrocytic anemia[56–58] neither anemia nor steatorrhea was altered by folic acid therapy in the majority of reported cases.[53,59]

It is clear from these reports that the steatorrhea of tropical sprue does not respond as readily to administration of liver or folic acid as do the symptoms or the hematologic defects. However, the studies mentioned measured fecal fat only over a period of weeks. As will be discussed later in this report in relation to other forms of therapy, it may require several months before steatorrhea decreases significantly even though a symptomatic response may occur within days. Perez-Santiago and Butterworth[60] found that in about one-third of patients with tropical sprue treated with folic acid over a period of years, there was continuing trouble with diarrhea and in 90 per cent of those steatorrhea persisted. Half of the patients who became asymptomatic on treatment still showed abnormal absorption tests.

Thus, long term studies show much more improvement in intestinal absorption than studies performed after a few weeks of treatment, but complete return to normal absorption is found in less than half of patients with tropical sprue treated with liver or folic acid. In non-tropical sprue and celiac disease liver and folic acid therapy have not been clearly shown to produce any improvement other than correction of anemia when this is due to folic acid deficiency.

Other substances which are effective in the treatment of pernicious anemia have also been found to cause hematologic remission in tropical sprue. Studies of intestinal absorption have not been reported, but in general symptomatic responses have been less dramatic than those to treatment with folic acid. Thymine[61] and folinic acid[62–64] give a less complete hematologic response, even with massive doses, while vitamin B_{12} [65–67] is as effective as folic acid when given parenterally but is ineffective when given by mouth.[68,69]

Current opinion[70] is that tropical sprue may be treated effectively by any of the following: folic acid, 15 to 20 mg. daily for two weeks, then 2.5 to 5 mg./day orally; vitamin B_{12}, 15 μg. daily intramuscularly for two weeks then 15 μg. every second day for two weeks, then 15 μg. every two weeks; or liver extract, 15 U.S.P. units intramuscularly for two weeks, then 15 units every second day for two weeks, then 15 units every two weeks.

Of these, orally administered folic acid is the treatment of choice since oral medication is best suited for long term management. In the unusual patient with tropical sprue whose illness includes neurologic manifestations, it

is preferable to use vitamin B_{12} or liver extract to avoid progression of the neurologic lesion which may follow folic acid therapy.

ADRENAL STEROIDS

The emaciation, postural hypotension, pigmentation and abnormalities of water[71] and electrolyte[72] metabolism commonly found in patients with intestinal malabsorption suggest the possibility of adrenal insufficiency. Thus, it was not surprising, in spite of the observation of a normal eosinophil drop in response to epinephrine in patients with sprue,[73] that adrenal cortical steroid therapy was undertaken in intestinal malabsorption. In 1951 reports appeared from the laboratories of Adlersberg,[15] Almy,[16] Astwood[18] and Pollack and Pollack.[17] In each study administration of ACTH, 50 to 100 mg. daily, or cortisone, 50 to 100 mg. daily, usually produced weight gain, loss of diarrhea and loss of deficiency symptoms such as edema or tetany. Taylor, Wollaeger and Comfort,[20] Badenoch,[19] Cooke,[74] Jones,[75,76] Drenick, Havolboll and Halstead,[77] Mackay and Volwiler[78] and Finlay and Wightman[79] measured improvement in fecal fat, nitrogen and calcium losses. Except for those of Jones and Culver[76] and Finlay and Wightman[79] these studies were of a few weeks' duration and it was found that the improvement in steatorrhea was short of a complete return to normal. However, in the longer studies fecal fat losses were reduced to normal over a period of months. Again one notes the slow return to completely normal fat absorption and the possibly false impression of incomplete response based on short term studies.

Although the response to administration of ACTH or exogenous adrenal steroids is reasonably consistent, relapse usually follows soon after withdrawal of steroid therapy, just as it does after discontinuing gluten-free diet in celiac disease or non-tropical sprue. Responses have been reported in celiac disease[80] and Whipple's disease.[21-23] Steroid therapy may produce more rapid response in severely ill patients with non-tropical sprue but the potential complications of long term therapy make it less satisfactory than gluten-free diet for maintenance. In some patients combined gluten-free diet and steroid therapy may be desirable,[79] although we have not yet found it necessary.

ANTIBIOTICS

In 1938 Rogers[81] reported a single patient with tropical sprue in whom stomatitis and diarrhea improved after treatment with Prontosil.® During the war it was repeatedly observed[39,82-85] that sulfaguanidine was often effective in stopping diarrhea in military patients with sprue in India. The effect was usually temporary and diarrhea returned when it was stopped.[85] Since that time, Anderson et al.[24] and French and associates[25] have treated patients with tropical sprue evacuated to England with antibiotics and disappearance of anemia, symptoms and steatorrhea were observed. Some of these patients were treated first with a gluten-free diet and many had received folic acid therapy. They were felt to be resistant to these measures, but in view of the slow response of steatorrhea to all forms of therapy it is possible that part of their improvement was a delayed response to previous therapy.

The importance of infection in primary malabsorption is not clear. It is traditionally stated that in this group of diseases bacteria invade the small intestine, but careful studies by Nadel and Gardner[86] and Anderson and Langford[87] have failed to show abnormal jejunal bacterial flora. Careful studies of ileal flora have not been carried out in patients with primary malabsorption. Sammons et al.[88] have isolated a streptococcus fecalis which synthesized fat in one patient, but this finding has not been duplicated. In patients with tropical sprue Black, Bound and Fourman[50] and Asenjo et al.[89] found that on a fat free diet fat excretion fell to normal levels. It would appear that endogenous fat excretion or bacterial synthesis of fat are not responsible for increased fecal fat in the usual patient with tropical sprue.

The importance of antibacterial therapy in primary malabsorption is not yet clearly defined.

GLUTEN-FREE DIET

In 1949 Dicke[12] and Weyers and van de Kamer[90] reported that when wheat products

were removed from the diet of patients with celiac disease anorexia, vomiting, abdominal pain and diarrhea disappeared, and the patient gained weight and began to grow. When wheat was refed, anorexia and diarrhea returned, weight decreased and growth ceased. Dicke, Weyers and van de Kamer[13] then showed, with careful and prolonged fat balance studies, that wheat flour, rye flour and oats increased both symptoms and steatorrhea in patients with celiac disease in whom remission had been induced by a diet free of these grains. Wheat starch, corn (maize), rice and potatoes were well tolerated by these patients. Further studies[91] showed that, in contrast to wheat starch, wheat protein (gluten) caused exacerbation of symptoms and steatorrhea when 14 gm./day was fed to two patients with celiac disease in remission. Gliadin, the 50 per cent ethanol-soluble fraction of wheat gluten, similarly produced steatorrhea when 3 to 5 gm./day was fed.

One of the characteristics of gliadin is its high glutamine content. When van de Kamer and Weyers[92] fed 2 to 4 gm. of free glutamine daily for six weeks to patients with celiac disease in remission no reaction occurred. When the glutamine of gliadin was hydrolyzed to glutamic acid by boiling for forty-five minutes with 1N HCl, the resulting protein permitted a remission when substituted for gliadin in the diet of patients with active celiac disease. These authors suggest that the untoward effect of wheat gluten is due to the proportion of glutamine bound in the protein. They support this with a table showing a parallelism between the ratio of amido nitrogen to non-amido nitrogen of foods and the reaction to the foods of patients with celiac disease. Thus, in meat, fish, potato, rice, corn and dairy products, amino acids containing an amido group (glutamine and asparagine) account for less than 11 per cent of the amino acid residues of the protein. Oats, with 13 per cent of the residues glutamine, and barley, with 14 per cent, produce mild symptoms and steatorrhea while rye flour (17 per cent), wheat flour (23 per cent), wheat gluten (27 per cent) and gliadin (33 per cent) produce increasingly severe reactions. Frazer[93] found that after digestion by pepsin and trypsin *in vitro* wheat gluten still produced deleterious effects in patients with celiac disease. A water soluble autoclaved peptide fraction of this digest still showed the effect but this was destroyed by further digestion with an extract of pig's intestinal mucus membrane.

Weyers and van de Kamer[94] gave 350 mg. of gliadin/kg. body weight and measured blood glutamine content. In normal subjects the rise in blood glutamine ranged from −13 to +40 per cent with a mean of +15 per cent. In patients with celiac disease the rise ranged from 55 to 193 per cent above the fasting value, averaging 104 per cent. In patients with celiac disease on a gluten-free diet the rise averaged 91 per cent and a few patients fell in normal range. Fasting levels in patients with celiac disease were lower (6 mg. per 100 ml.) than those in normal subjects (8.4 mg. per 100 ml.) while peak levels averaged higher (9 vs. 11.2 mg. per 100 ml.). Alvey, Anderson and Freeman[95] and Payne and Jenkinson[96] confirmed this difference between patients with celiac disease and normal subjects, although they found enough overlap between normal subjects and patients with celiac disease to prevent use of this abnormality in a diagnostic test.

The clinical response of patients with celiac disease to a gluten-free diet was confirmed in 1952 by Anderson et al.,[97] Sheldon and Lawson[98] and Soderhjelm[99] and subsequently by a host of others. Statements of skepticism have been published[100−102] but have not been supported by case reports or studies of intestinal absorption. Studies of fat balance published by several investigators[97,99,103−105] show decrease in fecal fat losses to or almost to normal within six weeks. Data on growth and residual symptoms is less extensive. The work of Ross et al.[106,107] implies that with the exception of a small portion of patients (7 per cent) who show no response to withdrawal of gluten, and are presumed to have a different disease, essentially all patients with celiac disease show a response to a gluten-free diet manifested by disappearance of symptoms and steatorrhea, weight gain and growth. This is quite in contrast to the chronic course of celiac disease prior to use of a gluten-free diet.[108,109] Refeeding wheat or wheat gluten, which is well toler-

TABLE I
Effects of a Gluten-Free Diet*
(Primary Malabsorption)

Patient	Control	1 wk.	2 wk.	3 wk.	4 wk.	2 mo.	3 mo.	4 mo.	6 mo.	9 mo.	12 mo.	15 mo.
						Fecal Fat						
D. S.	49 (72)		35 (39)			46 (53)						
B. W.	35 (56)			22 (15)		20	11 (9)		4 (3)			4 (3)
R. B.	52 (61)					28			17		16	
M. E.	64 (48)	27 (18)	19 (10)		18			4 (4)				
M. Mc.	22 (30)	22 (29)			9			7 (10)		9 (14)		
M. S.	49 (39)	41 (36)				25 (20)						
M. Mo.	33 (35)	18 (19)		28 (34)		13 (17)	22 (24)	7 (7)				
M. B.	15 (21)	13 (13)	15 (13)						7 (9)	7 (9)		
E. M.	26 (35)	45 (26)	33 (25)	37								
M. H.	31 (22)	28 (19)										
						Fecal Nitrogen						
D. S.	5 (26)		2 (8)			6 (36)						
B. W.	6 (32)			3 (13)		3	2 (12)		2 (11)			1 (7)
R. B.	6 (30)					4			3		4	
M. E.	5 (41)	2 (16)	3 (18)		4			1 (10)				
M. Mc.	3 (25)	3 (23)			3			0.3 (2)		2 (20)		
M. S.	4 (27)	3 (21)				0.4 (3)						
M. Mo.	3 (24)	1.6(13)		3 (28)		2 (22)	3 (23)	1 (11)				
M. B.	2 (14)	3 (18)	4 (27)						4 (33)			
E. M.	5 (42)	11 (54)	7 (30)	8								
M. H.	4 (27)	4 (24)										

* Fecal fat and fecal nitrogen are expressed as gm./day and as (per cent of intake). Figures represent the average for a five-day period. Outpatient collections are expressed only in gm./day. Fat was determined by a modification of van de Kamer's technic.[120,121] Nitrogen was determined by the Kjeldahl technic.[122]

ated by normal children[97] produces relapse[12,13,90–92,103–105,109] in patients with celiac disease.

It is traditionally felt that celiac disease disappears by the early teens, leaving no residual absorptive defect. However, recent studies have shown mild (up to 14 gm./day) steatorrhea in some[108] or most[109] adults who have "recovered" from celiac disease. Bone age was subnormal in one-third, weight averaged 12 per cent and height 7 per cent below the average for age.[109] Thus, children with celiac disease should be continued on a gluten-free diet at least until growth is complete and until symptoms do not recur when the diet is stopped. In many instances this means continuing the diet into adult life.

Adults with primary malabsorption (nontropical sprue, idiopathic steatorrhea) were reported improved symptomatically on a gluten-free diet by McIver,[14] who also measured decrease in steatorrhea. Since these reports in 1952, further reports of symptomatic improvement[110–118] and decrease in steatorrhea[112–118] have appeared.

Just as the gastrointestinal response of patients with severe native tropical sprue to administration of folic acid is slower and less complete than the response of military patients with milder tropical sprue, the response of adults

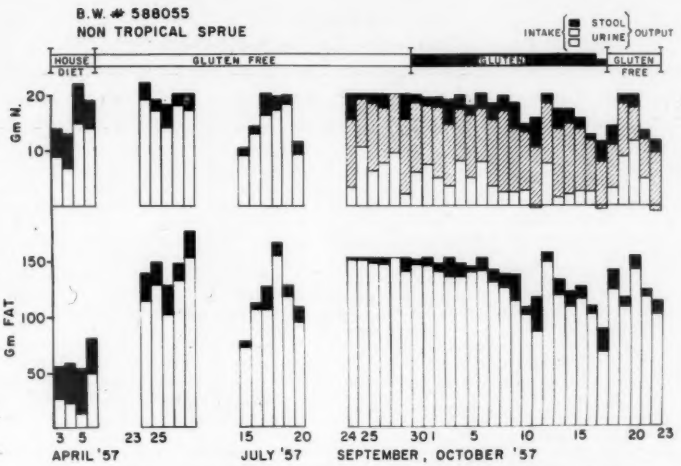

FIG. 1. Effect of feeding gluten on non-tropical sprue in remission.

with non-tropical sprue to a gluten-free diet is less dramatic than that of children with celiac disease. While children with the milder steatorrhea of celiac disease respond well in two to six weeks, the adult with longer standing, more severe steatorrhea may require two to six months before improvement in steatorrhea is maximum.

RESULTS OF MANAGEMENT

The experience of our group is similar to that of others and is presented as an illustrative example. It is based on ten adult patients with primary malabsorption treated with a gluten-free diet at University Hospital between January 1957 and March 1959. In each case the clinical picture was that of primary malabsorption; roentgenograms of the small intestine showed the abnormal pattern first described by Snell and Camp.[119] Steatorrhea of over 10 gm./day, flat vitamin A and flat glucose tolerance curves were noted in all. In each case findings of biopsy of the small bowel by the Shiner tube were characteristic of primary malabsorption with thickened, shortened villi and increased cellular infiltration in the stroma. Methods used for absorption tests are described elsewhere.[120]

When the gluten-free diet was started in a patient with primary malabsorption, the patient usually began to feel better within a short time, from one or two days to a week. This improvement was characterized by increased appetite, decreased diarrhea and increased sense of well-being. The irritability and emotional lability which characterize many of these patients disappeared rapidly. Diarrhea disappeared completely within four to eight weeks. In spite of this symptomatic improvement, fecal fat and nitrogen often remained unchanged for several weeks, then gradually improved over several months. This gradual improvement in steatorrhea and creatorrhea is documented in Table I. In every patient who followed the gluten-free diet carefully for a period of six months fecal fat decreased below 10 gm./day and in most became completely normal. Exceptions in Table I were patients D. S. and R. B. who failed to follow a completely gluten-free diet. In these two patients strong psychologic and sociologic reasons for not wishing to get well were suspected. Neither patient complained about the gluten-free diet and it is not believed that their failure to follow it carefully is evidence of failure of patient acceptance. One patient (M. S.) felt so well that she was unwilling to come back for studies after the first two months on the gluten-free diet. The effect of feeding wheat[12,13,91,97,103,105,106,112] rye[13,105] or oats[3,91,105] have been repeatedly demonstrated although the effect has not always been noted with oats.[113] Figure 1 illustrates the effect on fat and nitrogen losses when gluten

TABLE II
Effect of Gluten-Free Diet on Body Weight*

Patient	Control	1 wk.	2 wk.	3 wk.	4 wk.	2 mo.	3 mo.	4 mo.	6 mo.	9 mo.	12 mo.	15 mo.
D. S.	67	68	75	70	77			80				
B. W.	90	91			104	103	106		109			
R. B.	112							132	136	134	136	
M. E.	99	103	106		115			119				126
M. Mc.	103				121		122			134		
M. S.	87	92							129	128		
M. Mo.	103		113			118	116	119		119		125
M. B.	64	67	65	70	72	97	102	105	107		103	
E. M.	119	120	127	129								
M. H.	80	83	87	95	101	118	126					

* In pounds.

was fed to one of our patients (B. W.). Symptoms included loss of well-being, irritability, generalized abdominal discomfort, loss of appetite and increased frequency of stool without frank diarrhea. Symptoms appeared as soon as gluten was fed but it was two weeks before fecal fat and nitrogen increased significantly in patient B. W. or in a second patient, M. B., to whom gluten was also fed. Fecal nitrogen losses were abnormal in all patients before therapy and returned to normal at essentially the same rate as fecal fat.

Gain of weight began as soon as appetite increased. As summarized in Table II, by the time fecal fat and nitrogen returned to normal, weight was usually at preillness levels or above. During the period of weight gain appetite tended to be increased and was often ravenous. After six months to a year appetite decreased but it was commonly necessary to voluntarily control intake of food to prevent excessive weight gain and obesity.

General and specific signs of dietary deficiencies improved rapidly with a few exceptions. Serum albumin and cholesterol (Table III) rose rapidly, paralleling the gain in weight. Serum albumin and cholesterol are also low in badly nourished patients with secondary malabsorption or pancreatic insufficiency. They rose with successful therapy and are considered the manifestation of a poor nutritional state rather than a metabolic defect specific for primary malabsorption. Edema, paradoxically, sometimes disappeared promptly but sometimes increased when it had previously been mild. When edema increased with therapy, it tended to remain for several months. Abdominal distention remained prominent for many months in patients who showed an excellent response in other ways. Characteristically this distention increased during the day and early evening and decreased at night. Usually it was accompanied by increased urine volume at night.[71]

Of the specific nutritional deficiencies, hypocalcemia was the most prominent. This was manifested by tetany. When it was present, certain precautions were necessary in the management of these patients, since several factors are commonly present in the patient with malabsorption which decrease the tendency for tetany. Disruption of these factors may increase tetany or produce cardiac arrest in tetanic contraction. The low serum albumin level results in less binding of calcium to albumin and a higher proportion of ionized calcium. Low serum albumin does not cause symptoms which require emergency treatment and the use of intravenous albumin is contraindicated in the presence of tetany. Hypoka-

TABLE III
Effect of Gluten-Free Diet on Serum Albumin and Cholesterol*

Patient	Determination	Control	1 wk.	2 wk.	3 wk.	4 wk.	2 mo.	3 mo.	4 mo.	6 mo.	9 mo.	12 mo.	15 mo.
D. S.	Albumin	1.4		1.7	3.5								2.1
	Cholesterol	84		141									
B. W.	Albumin	2.8			4.7			4.5		3.8			
	Cholesterol	145			185			216		155			
R. B.	Albumin	3.3									4.6		
	Cholesterol	101						195			260		
M. E.	Albumin	3.8							5.2				
	Cholesterol	47							188				
M. Mc.	Albumin	4				4.8			4.8		4.7		
	Cholesterol	95				115			183		191		
M. S.	Albumin	3.9					4.9						
	Cholesterol	78					192						
M. Mo.	Albumin	3.7				4.8			4.3				5.2
	Cholesterol	143							137				194
M. B.	Albumin	1.4		3.4		4.2		5.7			7.4		
	Cholesterol	89		136							163		
E. M.	Albumin	4.4											
	Cholesterol	240											
M. H.	Albumin	3.9	4.1				5						
	Cholesterol	163	157				177						

* Serum albumin is expressed in gm./100 ml.; cholesterol in mg./100 ml.

lemia, here due to excessive losses of potassium in stools, also makes the patient less sensitive to hypocalcemia. On several occasions too vigorous replacement of potassium without equally vigorous parenteral calcium therapy resulted in precipitation of tetany. Acidosis increases ionization of calcium, so that parenteral alkali therapy on one occasion resulted in tetany. These electrolyte imbalances seldom cause symptoms that require emergency parenteral replacement. In general, oral replacement is preferable. When, as in the acute distention with paralytic ileus occasionally seen in these patients, hypokalemia is a cause of dangerous symptoms, potassium and calcium must both be replaced.

Deficiency of vitamin K was usually manifested by small decreases in prothrombin concentration. Prothrombin rose rapidly when the gluten-free diet was started. In most cases, prothrombin was not decreased enough to produce a bleeding tendency but when it was much reduced, this was rapidly corrected by parenteral administration of vitamin K. Fasting vitamin A levels were usually low but the only symptom due to lack of vitamin A was night blindness, which responded well to the vitamin A contained in the diet.

Deficiency of vitamin D along with the formation of unabsorbable calcium soaps in the intestine, plays a major part in producing the depletion of body calcium which results in osteomalacia and tetany. If a high fat diet is fed in order to increase absorption of calories, it is necessary to compensate for increased loss of calcium in the form of soaps. In the patient with primary malabsorption who responds well to one of the therapeutic regimens already mentioned, oral administration of vitamin D and calcium replacement are often the only long term medication added to the basic regimen.

Antianemic factors, folic acid, iron and vitamin B_{12} are commonly poorly absorbed in primary malabsorption. In most patients studied, absorption of both these substances improved on a gluten-free diet, but in two patients stores of iron were so slowly replenished that hemoglobin remained below 9 gm. per 100 ml. after three months of a gluten-free diet. In each patient absorption of Fe^{59} was essentially normal at this stage. In each,

anemia disappeared with parenterally administered iron therapy.

Early reports, such as that of Swan[129] considered that the gluten-free diets failed in adults when no improvement occurred in a few weeks. The transition in thinking is illustrated by the series of reports from Birmingham, England, wherein at first 5 out of 12 were reported to respond[112] and later 16 of 22[115,116] were found to respond with longer periods of therapy. In the combined series from Birmingham (16 of 22), New York Hospital (17 of 20)[113,114] and Ann Arbor (8 of 10) 80 per cent of patients have shown excellent response and at least four of the failures were in patients who failed to follow the diet carefully. Most patients in whom the diagnosis of non-tropical sprue is carefully established will respond to a gluten-free diet. Instructions and recipes are available[123,128] and are very helpful in making the diet attractive to the patient.

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Panel Discussion

DR. DAVID ADLERSBERG* (New York, New York): Dr. Butterworth has been quoted before as being interested in the study of the ultra structure of the jejunum in normal patients, in normal control subjects and also in patients with the malabsorption syndrome. Dr. Butterworth has worked with the San Juan team in Puerto Rico for many years and then at Walter Reed Hospital. At present he is in Birmingham and was kind enough to join us. Dr. Butterworth, may I ask you to tell us something about this work?

DR. CHARLES E. BUTTERWORTH, JR. (Birmingham, Alabama): I think the many interesting papers and comments have borne testimony to the many facets of sprue, and to the interest that many of us have in this subject.

The appearance of the jejunal specimen in sprue has already been covered by Dr. Adlersberg, but I would like to discuss biopsy specimens.

An electron photomicrograph made by Dr. Hartman at the Walter Reed Hospital, demonstrated a goblet cell in the act of discharging its mucus, the brush border, and of course the nuclei and the interdigitated cell borders, which place each cell in intimate contact with it neighbor. The brush border itself is made up of microvilli. (In studying biopsy specimens we find that in normal people there are about 60 microvilli in a cross section of a cell, making approximately 3,600 per cell. These measure approximately a tenth of a micron in diameter and micron in length.)

In (untreated tropical sprue) you will see the columnar cell nuclei. Microvilli are not apparent. There is a vacuole where the brush border should be. These may also be seen in light microscopic sections. (In untreated tropical sprue the brush border seems to be grossly distorted if not entirely absent.)

If we remember that there are about 3,600 microvilli per cell it will give us a fair indication of the order of magnitudes of difference. We can calculate that the little top of the microvillus would have a surface area approximately 0.008 square micron, and that using the formula for the surface area of a cylinder, we can find that the sides would have a surface area of 0.314 square micron, for a total surface of 1 microvillus of approximately 0.322 square micron. This feature of the cells will endow the cell with a surface area that is approximately twenty times greater than the surface area would be if the surface were flat and plane. And further, we might state that if the microvilli are shortened by one-half their length, the surface area would be reduced by one-half, just as surely as if one-half the intestine has been resected. I would like to place these preliminary observations before you to indicate the importance which may be present in the factor of cell surface for the absorption mechanism.

DR. ADLERSBERG: This is certainly an intriguing and important approach to the problem. I do not know whether this picture is reversible. Could you say anything about that? Did you see any changes brought about by therapy with gluten-free diets or steroids?

FROM THE FLOOR: We have had an opportunity to study two patients, both before and after the institution of a gluten-free diet, and the changes in the brush border were even more striking than those described by Dr. Butterworth. In the patients with sprue there were practically no microvilli whatever before they were given a gluten-free diet. After that, fat absorption and all clinical evidences of sprue disappeared. The brush border appeared perfectly normal.

DR. ADLERSBERG: Dr. Victor Herbert has developed a very interesting technic of studying the presence or absence of the intrinsic factor in the gastric juice. I would like him to tell us something about that.

DR. VICTOR HERBERT (Boston, Massachusetts): Our work has been concerned with mechanisms of vitamin B_{12} absorption and has been carried out primarily with rat and human liver and intestine *in vitro* and *in vivo*.

We have found that the initial uptake of vitamin B_{12}, by either intestinal mucosa or by liver cell surface, is apparently a physical phenomenon, it is not metabolic, and it is calcium-dependent and reversible by EDTA.

We have used this simple system, working mainly with rat liver slices or homogenates, as an "assay" for intrinsic factor, and so far it has been uniformly successful in our hands in patients with achlorhydria. We have used gastric juice from patients with pernicious anemia, from patients with the malabsorption syndrome, and from normal persons with achlorhydria. We find that gastric juice from patients with pernicious anemia has no effect on vitamin B_{12} uptake by liver slices or liver homogenates, whereas gastric juice from patients with the malabsorption syndrome or from normal persons markedly enhances such uptake.

We hope that this will prove, in fact, to be an assay for intrinsic factor. We cannot call it such, because intrinsic factor has never been purified. We do not even know what intrinsic factor is. We believe, as most people do, that it is probably a mucoprotein, but we have to agree with Dr. Glass and with others that it may be nothing more than a prosthetic group.

We might mention that the calcium effect may play a role in the intestinal malabsorption of vitamin B_{12}

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* Deceased.

Discussion at Symposium on Absorption Mechanisms and the Malabsorption Syndrome, under the sponsorship of The National Vitamin Foundation, Inc., March 3, 1959, New York, New York.

in certain patients with malabsorption syndrome. We reported, at the last symposium of the National Vitamin Foundation, that sodium phytate, a calcium chelating agent, would markedly inhibit vitamin B_{12} absorption in normal persons. In the Scandinavian literature Grasbeck and co-workers have reported a similar phenomenon observed with EDTA.

Furthermore, they made a striking observation, which was reported in *Lancet*, in a few patients with steatorrhea, especially in one such patient. This patient, who was almost totally unable to absorb vitamin B_{12}, when given calcium lactate orally, absorbed vitamin B_{12} in completely normal fashion. The same phenomenon to a slight degree was noted in their other patients. They concluded that the mechanism of vitamin B_{12} absorption was in fact calcium-dependent, and that the inhibition of vitamin B_{12} absorption in at least some patients with the malabsorption syndrome, is due to the fact that the fatty acids tie up calcium in the intestine, precipitate as calcium soaps, and are excreted in the stool, thereby making it impossible for vitamin B_{12} to be absorbed.

DR. ADLERSBERG: The group at Cornell, as you know, has been interested for many years in metabolic and balance studies, somewhat similar to those presented by Dr. French. Dr. Sleisenger, would you like to discuss this?

DR. MARVIN H. SLEISENGER (New York, New York): I appreciate very much the invitation to discuss the upshot of seven years' work.

The group at The New York Hospital has treated non-tropical sprue or adult celiac syndrome for a number of years, particularly with the elimination of gluten and gliadin from the diet.

I think is it extremely important to emphasize the concept which Dr. Cooke placed before us of calling this syndrome adult celiac disease. I have now come to the point where I am willing to say that this disease represents a gluten enteropathy. Three years ago, when I presented our metabolic data in Atlantic City on a group of such patients, I was appropriately hesitant and conservative about it. The corroboration of our results by other groups with this form of therapy now permits greater certainty of this concept. The importance of the role of gluten in non-tropical sprue is further emphasized by several observations: (1) Elevated excretion of urinary 5-hydroxy indole acetic acid in patients with non-tropical sprue in relapse, or even mildly symptomatic on steroid therapy with fall to normal levels upon successful institution of dietotherapy. (2) Observations of Girdwood that ingested folic acid is improperly metabolized in non-tropical sprue as evidenced by reduced excretion of urinary metabolite. The defect apparently continues despite correction of anemia in these patients with gliadin elimination. (3) Findings of Weijers and van de Kamer that, after feeding gliadin to celiac children, there is an abnormal rise in blood glutamine. (4) The evidence for disturbance of ascorbic acid metabolism in non-tropical sprue.

Dr. Adlersberg stated that during remission patients still had diminished absorption of fat and fat-soluble vitamins. This has not been the case in patients treated with the gluten-gliadin-free diet. I presume his patients were receiving adrenocorticosteroid therapy. This has also been the experience, as you know, of the Mayo Clinic group and of others who have studied metabolism of these patients during steroid therapy.

That the difference between tropical and non-tropical sprue is superficial, I would disagree. I think the weight of the evidence, both clinically and metabolically, not to speak of biochemically, is that we are dealing with two different entities that have the same or similar clinical appearance.

A brief report of the results of a metabolic study made in 1955 on a patient with non-tropical sprue follows:

The patient was a thirty-seven year old woman, who had had a celiac disease from the time she was two years of age. During the control period the patient absorbed only 54 per cent of fat which was administered. Although the patient was in positive nitrogen balance, the urinary creatinine excretion was low, and the patient weighed only 74 pounds. She was then placed on a gluten-gliadin-free diet, and within a matter of three days there was some increase in fat absorption, although not, I am sure, great enough to be statistically significant. Nitrogen retention also began to increase; and in a further period of three days, fat absorption rose to 68 per cent, and there was further increase in positivity of nitrogen balance. The patient was studied again two months later, at which time fat absorption was 94 per cent, and although positivity in the nitrogen balance was reduced, the ratio between the urinary excretion of nitrogen and its fecal excretion was reversed toward the normal. At the same time, the patient's urinary creatinine, a reflection of nitrogen metabolism, had more than doubled and she had gained 35 pounds.

On administering a diet which contained wheat, oat or rye products, the patient began within a short period to have steatorrhea.

We may summarize in one individual the biochemical changes noted in twenty-four of our twenty-six patients. This was a young woman in whom a rather severe picture of the so-called malabsorption syndrome developed due to adult celiac disease. This young woman was cachectic, weighed a little over 39 kg., and was having foamy diarrhea. Blood albumin was only 1.9 gm. per 100 ml. and prothrombin time was elevated. The serum cholesterol also was very low. She was placed on a gluten-gliadin-free diet, and despite loss of weight due to mercurial diuresis, this patient gained about 15 pounds during the six weeks that the diet was given. At the end of this time the albumin was normal, as were all the parameters which depend upon normal fat absorption such as serum calcium, carotene, cholesterol and plasma prothrombin time.

Last year, at the annual meeting of the Association

of American Physicians in Atlantic City, we reported on twenty patients with non-tropical sprue, seventeen of whom had responded to dietotherapy. We now have twenty-six patients and in all but two, therapy has been successful. Of these two, one is suspected of having another disorder, and the other is not adhering to the diet closely.

The diarrhea, which was moderate to severe in all instances, remitted; the change to normal usually occurred within seventy-two hours, but occasionally it required several weeks. The weight loss, which had been remarkable in these patients, was corrected in all instances. The weight increases were so dramatic that about 25 per cent of these patients eventually restricted their caloric intake.

The duration of sustained remission, without any other form of therapy, is now twelve months longer than the longest one, so that it is almost six years.

I think the biochemical implications are more important than the clinical results. Some other work has been carried out by Dr. Kowlessar in our laboratory which is quite interesting; he may have an opportunity to present that work to you.

DR. ADLERSBERG: I am struck by the fact that of your seventeen patients sixteen were female and one male. Is this correct?

DR. SLEISENGER: Of our total of twenty-six patients, twenty-three are female.

DR. ADLERSBERG: Is this not a very high ratio of females?

DR. SLEISENGER: Yes.

DR. ADLERSBERG: Dr. Kowlessar, would you like to continue this discussion?

DR. O. DHODANAND KOWLESSAR (New York, New York): Like most investigators, one becomes interested in the reason why things occur in medicine, i.e., the abdominal distention, the nausea and diarrhea that one sees frequently in patients with non-tropical sprue. Because of this, we began a study of the urinary excretion of 5-hydroxy indole acetic acid in non-tropical sprue, and these are the data at the present time.

In our patients with non-tropical sprue, prior to dietotherapy, one can see levels of 5-hydroxy indole acetic acid ranging from 11 to 17 mg. per twenty-four hours. In our normal subjects, the values ranged from about 1 to 6 mg. per twenty-four hours. These patients also had abnormal fat excretion at this time, and were not considered in remission.

Other diarrheal states with steatorrhea that we have had the opportunity to study include ileojejunitis, regional enteritis and pancreatitis with pancreatic steatorrhea. Only our patients with malignant carcinoid and those with symptomatic non-tropical sprue had elevated levels of 5-hydroxy indole acetic acid. The significance of elevated levels of urinary 5-hydroxy indole acetic acid is under investigation.

DR. ADLERSBERG: There are a number of questions here, which are extremely interesting. Dr. Cooke, what is known about the interrelations between vitamin B_{12} and folic acid, iron and folic acid, iron and vitamin B_{12} absorption? Can you answer that in one minute?

DR. W. TREVOR COOKE (Birmingham, England): I do not think I can answer in a minute but I can add a certain amount of confusion to the problem. If you follow the blood levels of folic acid of a patient with vitamin B_{12} deficiency and administer a dose of vitamin B_{12}, there is a gradual increase of folic acid levels rising to a maximum ten to twenty days later, the increase being as much as tenfold. If you also measure the urinary excretion of folic acid following test doses of folic and administer vitamin B_{12} there is a fall in the urinary excretion of folic acid (*Clin. Sc.*, 17: 693, 1958). A rise of folic acid blood levels is seen following severe hemorrhage or in response to the administration of hematinics. If an injection of 5 mg. of folic acid is given to a patient with pernicious anemia in relapse, insufficient to cause any adequate hematological response, only about 1 mg. is usually excreted in the urine. However, a further dose of 5 mg. will show the patient to be fully saturated. It is clear as far as pernicious anemia is concerned that deficiency of folic acid is not the primary defect. Also if the levels of folic acid in the marrow of patients with pernicious anemia and with adult celiac disease are considered they are not outside the realms of normal although the concentrations are rather on the low side; but when taken in conjunction with the increased volume of bone marrow in these patients then the absolute amount of folic acid is considerable

My colleagues and I believe that there must be an upset in folic acid metabolism and we have demonstrated this elsewhere. We do not know how vitamin B_{12} affects it; ascorbic acid is involved for vitamin B_{12} administration to patients with megaloblastic anemia restores the fasting ascorbic serum levels to normal and also the rate of disappearance of ascorbic acid from the plasma. We have not investigated whether folic acid administration will do the same.

FROM THE FLOOR: What do you mean by your folic acid?

DR. COOKE: Pteroylglutamic acid activity.

FROM THE FLOOR: I supposed you were measuring the net effect of about six different substances there, which may have varying metabolic effects.

DR. COOKE: I will accept that; but I do not think anybody can get any closer at the moment. All I can say is that the net total of the things do what we show there, pteroylglutamic acid activity.

DR. ADLERSBERG: Here is another question. "Does Dr. Cooke relate folic acid blood levels to body build if his standard dose of 5 mg. is used? This might explain some of his different expressions, percentagewise."

DR. COOKE: The answer is no. I do not think it makes the slightest bit of difference in the technics used, and you cannot relate it to any body build in our tests.

There is one further point that should be made and that is tissue unsaturation which may be found in

any malnourished patient as judged from urinary excretion of folic acid following intramuscular injection and which does not present clinical manifestations of folic acid deficiency, is not the same as folic acid malutilization which is an entirely different problem.

FROM THE FLOOR: How do antibiotics work in malabsorption?

DR. FRANK H. GARDNER (Boston, Massachusetts): I do not know. We see more and more of these patients respond to therapy with antibiotics; and a large number of patients with tropical sprue have responded, as measured by decreased fecal fat excretion. I am anxious to see some of these patients given massive amounts of ascorbic acid, because I wonder if we actually are not seeing a true intraluminal bacteria competition for vitamin C. If we could eliminate the bacterial metabolic needs *per se* without antibiotics, could we not study the problem by leaving the bacterial flora intact and provide adequate nutrition, so that it would not be competitive for nutrients?

FROM THE FLOOR: In other words, your explanation would be based on the same principles as have been used to explain the effect of antibiotic therapy in the blind loop syndrome, if I am correct.

DR. GARDNER: Yes,

DR. A. LEONARD LUHBY (New York, New York): My colleagues and I have been interested in the metabolism of amino acids which are dependent upon folic acid. This work was encouraged by Dr. Jukes and Dr. Broquist a few years ago.

We have studied particularly the degradation of formiminoglutamic acid to glutamic acid. This step requires folic acid. The absence of folic acid interferes with the normal degradation of formiminoglutamic acid to glutamic acid. The formiminoglutamic acid then accumulates and is excreted in the urine. Rats made folic acid-deficient by diet plus sulfonamides excrete excessive amounts of urinary formiminoglutamic acid. The quantity is in direct proportion to their folic acid deficiency. We have found that many patients with sprue and malabsorption syndromes also excrete considerable quantities of formiminoglutamic acid in their urine. If this can be taken as a criterion of folic acid deficiency, and there seems to be good evidence that this is so, then study of the urinary excretion of formiminoglutamic acid provides a direct biochemical means of assessing the folic acid nutriture of patients with sprue and malabsorption syndromes. A report of our findings has appeared (*Am. J. Clin. Nutrition*, 7: 397, 1959).

DR. ADLERSBERG: There are two questions we could probably answer together. Is there any feeling by the panel that the patient with sprue may do well to follow other than a gluten-restricted diet? What is the experience of the panel with gluten-free diets in conditions other than non-tropical sprue? Would you start, Dr. Cooke?

DR. COOKE: As a matter of clinical practice, I find it difficult at times to make a patient adhere to a gluten-free diet. I am always reminded of the number of patients that I have had for sixteen or seventeen years, who have had only a fortnightly injection of a crude liver extract, and yet have maintained good health, with all their biochemical abnormalities going strong, and leading a hard working life. When you have patients like that it is difficult to persuade them to abandon their hematinics and take to diet. If they are not feeling well then they should go on a gluten-free diet.

As far as the other conditions, secondary steatorrheas, are concerned, we have not made any exact studies but I have the impression that some diminution of the amount of diarrhea does result. In fact, if you are on a gluten-free diet you will find yourself becoming very constipated.

DR. ARTHUR B. FRENCH (Ann Arbor, Michigan): For some reason, our patients do not seem to have the trouble staying on a gluten-free diet that other people's patients do. We have a set of recipes that we make available to them, but I know that Dr. Cooke and others have similar recipes that their patients use.

Our diet is very strict. The reason for our success in keeping patients on the diet is not a less strict diet. Actually, in England most of the groups, I believe, do permit oats, which we do not permit. The reactions which have been shown to oats are relatively milder than those to rye, barley and wheat. Perhaps it does not make too much difference whether they have oats or not; but our patients do adhere very closely to the diet. We have had no trouble with keeping patients on the diet except for one girl, who had very striking psychologic problems, and for whom there were obvious reasons why she preferred to be in the hospital, and one other patient.

DR. ADLERSBERG: What about the use of gluten-free diets in other diarrheal conditions?

DR. FRENCH: In secondary sprue, there will be an occasional response, and certainly we have little else to offer these patients. Use steroids and see if they work, and certainly a gluten-free diet should be tried in any patient in whom nutrition is a problem. I would emphasize that in many of these patients it is not nutrition that is the problem.

For instance, in lymphoma or in tuberculosis or regional enteritis, in most cases the malabsorption is easily demonstrable but it is not the limiting factor in these patients. There we should work toward modifying the underlying condition rather than the malabsorption. But when nutrition is the limiting problem, I think a gluten-free diet should be tried, and if it does not work, it can be discontinued.

DR. GARDNER: We placed everybody on a gluten-free diet because we wanted to evaluate absorption. I think we ought to be quite frank and say that the clinical results are good, and that we want to study certain histochemical and hematinic reactions. I am sure that if I did not want to do this, I would not put them on a gluten-free diet unless they were unable to be maintained with the usual measures that have worked in the past.

DR. ADLERSBERG: What I have been impressed with during the past year or so is that patients with ulcerative colitis and also ileitis who had no evidences of malabsorption occasionally responded very strikingly to a gluten-free diet. Dr. Sleisenger, have you had any experience with the gluten-free diet in conditions other than celiac disease of the adult or non-tropical sprue?

DR. SLEISENGER: We have had some experience, but it is not enough to take any strong stand. We have treated several patients with regional enteritis and ulcerative colitis with this diet in, as Dr. Gardner would put it, a university hospital. Despite our enthusiasm, it did not work. Another patient, in whom there was a decrease in steatorrhea, turned out eventually to have a lymphoma. However, I would remind you that experimentally it is possible to induce not only diarrhea but also steatorrhea in animals by giving them gluten in the diet. And the fact that the elimination of gluten may ameliorate other diarrheal states does not detract from the specific role that a glutamine polypeptide may play in the adult celiac syndrome.

I would strongly disagree with Dr. Gardner about the choice of treatment in non-tropical sprue. Except in the most desperately ill patient, the choice of therapy is the gluten-gliadin-free diet. I think that the "usual measures" cannot in the long run be very dangerous, with the exception of prolonged steroid therapy.

I am sure you will have an opportunity to clarify this, for I think we should realize that even if a patient is receiving small doses of corticosteroids, 5 to 10 mg. daily, over a period of years there can be exceedingly detrimental and dangerous effects, particularly with reference to the skeletal system.

DR. ADLERSBERG: I certainly would disagree with your last statement. We have treated a considerable number of these patients on normal diets, which is a terrific advantage, with small doses of steroids over a period of many years. If you really do it with minimal doses, there is absolutely no danger, provided you watch your patient carefully. With doses of 5 or 7 mg. of prednisone daily or correspondingly small doses of Decadron,® say, 0.075 mg. per day, we have not seen any severe complications, even after several years of treatment.

One more question. Why does the absorption defect in tropical sprue persist in 50 per cent of patients after treatment has caused their symptoms to disappear? Do you think that symptoms disappear first and that if these patients are followed up long enough the results of the absorption tests will become normal, too?

DR. GARDNER: No, I do not think that the fat balance and other absorption studies will revert to normal in a patient who has had sprue long enough to have a severe megaloblastic anemia develop, and then responds hematologically. Certainly some of the patients studied in the Puerto Rican series, who have been under therapy for many years, still have abnormal tolerance tests and excess fecal fat.

In fact, I believe one of the first patients described by Dr. R. Suarez in the early use of folic acid was in 1947. Twelve years later she still had defects in absorption, despite having had an initial and sustained hematological response. I think if you can review the bowel biopsy reports that Dr. Butterworth and Perez-Santiago originally published on treated patients with sprue, you can understand that the jejunal mucosa in those patients was still abnormal despite years of therapy.

End of Symposium

Editorial

Diet and Atherosclerosis: Significance of Exceptions That Test the Rule

In the enormous literature on diet and atherosclerosis pouring out in increasing volume each year, there are two main refrains. One ignores nearly every factor but diet, and skips lightly over national statistics which do not fit on the curve relating coronary deaths to per capita ingestion of animal fat. The other emphasizes every factor except diet and concludes "there is much more work to be done before any conclusion can be drawn as to the relation of diet to arterial disease." Of course there is always more work to be done in clarifying any biologic problem, and it is obviously most desirable that much more information be obtained on species and individual responses to emotional stress, physical activity and dietary factors in relation to atherogenesis, thrombogenesis, and of both of these to plasma lipids. One way to throw light on this crucial problem is to investigate the apparent exceptions to the rule that in large cultural groups the coronary death rate is related to the intake of saturated fats.

It is, of course, irrelevant that there is a relation between intakes of protein and saturated fat, or even between the latter and per capita purchases of television sets, automobiles, or mink. There is clear-cut experimental evidence, in many species of warm-blooded vertebrates, that raising the intake of saturated fats raises plasma lipids and evokes atherogenesis and thrombogenesis. There is no evidence that such lesions can be evoked by high intake of protein, nor by exposing animals to television, carrying them in automobiles, or setting the cages on second-hand furs. The fact that rich people have more severe atherosclerosis than the poor was what led Ignatovski to feed egg yolk and butterfat to rabbits, since the intake of these foods has always been high in the diet of the rich and low in the diet of the poor. The fact that coronary disease is correlated to various indices of per capita wealth is not new, and is relevant only when experiment shows a connection between wealth and factors important in atherogenesis. This has been shown for cholesterol and fat.

Although experiments on animals first proved the importance of dietary lipids in determining the levels of plasma cholesterol and rates of atherogenesis, studies of human populations have not always confirmed the relationship between diets rich in animal, egg and dairy fats and the coronary death rates and plasma lipid levels.[1,2] When one compares death rates from coronary disease with diets on a national scale, one finds that the death rates rise from 6.5 per 10,000 per year, at age fifty-five to sixty-five in Japanese men, with almost no eggs and no dairy products in their diets, to 82 per 10,000 per year, in the United States, with its very high intake of these foods.[2] These countries seem to fit nicely on a slope relating animal fat intake to coronary death rate. So do many other countries, as emphasized by Keys.[3]

However, Yudkin[1] and Yerushalmy and Hilleboe[2] point out the striking exceptions to this relationship, and see in these a complete refutation of the idea that diet, and notably animal fat, plays a great role in atherosclerosis. Obviously, much more needs to be known about the life-long levels of fat ingestion in countries with low coronary death rates, such as France (12 per 10,000), or Holland, Denmark and Norway (under 25 per 10,000) where the intake of animal fat is relatively high.[2] These coun-

tries, and England with a coronary death rate of 45 per 10,000, also consume more fish, which has protective unsaturated fats, but this alone scarcely can be credited with the difference in coronary death rates. It is worth noting that groups with low coronary death rates, but high rates for cerebrovascular deaths, have low fat but high salt intake, and much hypertension. This has been striking in Japanese and in African Bantus.

One place to look for a possible explanation of these exceptions to the rule is in the rates of atherogenesis and in diets during adolescence. In America, as Holman[4] in New Orleans and Lober[5] in Minnesota have shown, atherogenesis in white males is most rapid between the ages of ten and twenty-five years. American dietary surveys show an incredibly high intake of dairy and egg fat by white males during these years, but no corresponding increase in either dietary fat or in atherogenesis in white girls.[6] While dietary surveys and studies of arteries in European adolescents have not been published, it is apparent in discussing the problem with European parents and pathologists that it is in this age group that a great difference from the American population is seen. Boys rarely have eggs or cream for breakfast or lunch, they consume little ice cream and do not drink milk. Their load of school work and modes of travel to school force them to a life which favors high caloric expenditure with a diet actually lower in animal fat than the diets of their parents (who have much more substantial mid-day meals).

Another factor is that of thrombogenesis. This can best be determined by the incidence of postoperative thromboembolism and of pulmonary emboli in autopsy material in various countries. (In several hundred autopsies of Negroes over forty years of age in St. Louis, Mo., myocardial infarction was found in 18 per cent, thromboembolic lesions in 22 per cent; in a similar number, matched by age and sex, in Uganda, the incidence was: thromboembolic disease in 2 per cent, infarcts in 0.4 per cent.[7]) If, as the English believe, thrombotic disease is more severe and atherosclerosis about the same in populations with high levels of coronary deaths as compared to others with lower rates, the incidence of thromboembolic disease should also be higher. How the way of life affects this mechanism and why it should be only one-third as frequent in France as in England demands further inquiry. Perhaps differences in habits of drinking, in the relation of the largest meal to activity or to sleep and in the use of salt rather than skill in cooking, play a part in the different rates on the two sides of the Channel.

The Latin aphorism "Exceptio probat regulam" is distorted when "probat" is translated as "proves" and not as "probes." Study of the exceptions which test the rule that diets and atherosclerosis are related undoubtedly will cast new light on the causes of the high rate of coronary disease in the United States. Perhaps it will turn out that psychologic factors, rather than differences in diet and physical activity, make the difference between a rate of 12 per 10,000 per year in France and 82 in America. This would confirm the belief of Kipling that the French are "lifted over all By the light sane joy of life, the buckler of the Gaul." But the readers of Celine and Simenon, of Anouilh and Camus, and of Jean Genet and Giono, take it for granted that both rural and urban Frenchmen are profoundly disturbed and frustrated by life, and that the stresses which may alter plasma lipid and thrombosis are as numerous and effective in France as in Manhattan or London.

WILLIAM DOCK, M.D.
College of Medicine
State University of New York
Brooklyn, New York

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Clinical Reports

Effects of the Composition of Dietary Fat upon Composition of Adipose Tissue

D. M. HEGSTED, PH.D.,* C. WHYMAN, M.S.,† A. GOTSIS‡ AND S. A. ANDRUS, M.D.§

THERE is abundant evidence to show that the fatty acid composition of the fat in various tissues, at least in some animals, can be influenced markedly by the composition of the dietary fat. Several reviews[1-3] have summarized the older literature. This area of investigation has received relatively little study in recent years although there has been a great deal of interest in fatty acid metabolism and the effects of dietary fat. Improvements in the alkaline isomerization method[4] for the measurement of polyunsaturated fatty acids and particularly the perfection of gas-liquid chromatography for the separation and measurement of fatty acids[5,6] will undoubtedly stimulate work in this field. Some of the recent studies have shown that the composition of the fat of human milk[7] reflects the composition of the dietary fat to a considerable extent. *Trans* fatty acids of dietary origin are apparently readily deposited in body fat of both man[8] and the rat[9] and polyunsaturated fatty acid content of the diet is reflected in the fat of eggs[10] and in the tissues of pigs, rats, chicks[11-13] and fish.[14]

We have had occasion to analyze the abdominal fat of hens fed diets containing different dietary fats. In spite of the evidence in the literature, we were surprised to find how accurately the fatty acid composition of the adipose tissue reflected the composition of the fat included in the diet. These findings may be of interest in view of the extensive studies now underway in all parts of the world upon the nutritional role of fat and fats of different composition, especially in coronary heart disease and atherosclerosis.

EXPERIMENTAL METHOD

Pullets were obtained from a commercial source when they were between three and four months of age. They were placed in individual cages and six received each of the experimental diets. The basic diet, similar to that used by Fisher et al.,[10] consisted of the following mixture: corn meal, 27.7 pounds; soybean meal (solvent extracted), 30 pounds; Solka-floc (Brown Company, Berlin, New Hampshire), 10 pounds; butyl fermentation solubles, 2 pounds; corn distillers solubles, 2 pounds; dried whey, 2 pounds; alfalfa leaf meal, 3 pounds; dicalcium phosphate, 4 pounds; trace mineral mixture (Limestone Corporation of America, Newton, New Jersey), 3 pounds; salt, 0.5 pound; vitamin B_{12} supplement, 0.5 pound; DL-methionine, 0.2 pound; vitamins A and D (10,000 A–600 D), 0.1 pound; choline chloride, 25 grams. Nine parts of this basal diet and one part of the appropriate oil were mixed to form the various rations fed. The oils used were coconut oil, safflower oil, olive oil, corn oil and

From the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts.

* Associate Professor of Nutrition; † Research Assistant; ‡ Senior Research Assistant; § Assistant Professor of Pathology.

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TABLE I

Survival, Production of Eggs, Mean Serum Cholesterol and Vascular Sudanophilia in the Various Groups

Category of Measurement	Group No. and Oil Fed				
	1 (Safflower)	2 (Coconut)	3 (Safflower-Coconut)	4 (Corn)	5 (Olive)
Survival to 15 weeks	6	6	4	6	6
Eggs per hen per week, 15 weeks*	2.79	3.59	4.41	4.13	4.48
Survival to 33 weeks	4	5	2	5	5
Eggs per hen per week, 33 weeks*	1.54	3.15	2.78	2.96	3.20
Mean serum cholesterol, mg. %	137	179	157	117	135
Degree of vascular sudanophilia	0–1+	2–3+	2–3+	2–3+	1–2+

* Calculated on the number of weeks each bird was alive.

an equal mixture of coconut and safflower oil. The birds were weighed at two-week intervals, egg production was recorded and serum cholesterol determination[15] made at intervals during the study. The studies were terminated after thirty-three weeks. The heart and aorta were opened *in situ*, excised and fixed in formalin. Following staining with Sudan IV, an estimation of the extent of gross lipid deposition in the aorta and brachiocephalic arteries was made using a scale of 1 to 4+. A sample of abdominal fat was removed, the methyl esters of the fatty acids prepared[16] for analysis upon the chromatograph (Pye Argon Chromatograph, Jarrell-Ash Company, Newtonville, Massachusetts). The column contained 20 per cent polydiethylene glycol/pentaerythritol adipate as the stationary phase supported upon 100–120 mesh Celite. The area of the principal peaks was calculated as the height multiplied by the width at one-half the height. The area of each peak was recorded as percentage of the total area measured. No attempt was made to measure or, in some cases, identify the minor constituents.

RESULTS

Survival was poor in the group receiving the coconut-safflower oil mixture (Table I). Two died before the fifteenth week and only two of

TABLE II

Comparison of the Fatty Acid Composition of the Dietary Fat and the Adipose Tissue

Fatty Acid	Group 1 (Safflower)		Group 2 (Coconut)		Group 3 (Safflower-Coconut)		Group 4 (Corn Oil)		Group 5 (Olive Oil)	
	Dietary Fat	Adipose Fat	Dietary Fat	Adipose Fat	Dietary Fat	Adipose Fat	Dietary Fat	Adipose Fat	Dietary Fat	Adipose Fat
Capric	...	...	3.7	Trace	2.2	...	...	...	...	...
Lauric	...	Trace	29.9	18.0	15.3	7.8	...	...	...	...
Myristic	...	0.9	21.9	14.7	10.5	6.6	...	0.8	...	0.5
Myristoleic	...	Trace	...	3.2	...	Trace	...	...	...	Trace
Palmitic	11.9	14.4	15.7	17.4	9.6	16.4	15.1	15.4	17.2	16.3
Palmitoleic	...	2.0	...	3.5	...	2.2	...	1.8	2.3	3.0
Stearic	4.6	6.9	4.3	4.2	3.3	6.3	2.4	5.2	4.2	5.4
Oleic	17.6	27.7	18.4	26.9	15.1	25.8	34.0	33.4	63.6	53.8
Linoleic	64.5	45.8	6.1	12.1	43.5	34.6	46.8	41.5	11.0	19.3
Linolenic	1.4	1.9	...	Trace	0.6	Trace	1.7	1.6	1.7	1.3
Unknown	...	1.6	...	...	...	0.6	...	0.5	...	0.4
Unknown	...	...	...	...	...	...	...	1.6	...	1.2

NOTE: All figures shown are percentages.

the original six survived the entire thirty-three weeks. The cause of death was not determined and whether this mortality is related to the diet is unknown. During the time the hens were alive they laid about as well as any of the other groups. Egg production was poor in the first group which received safflower oil. Again the role of the diet is problematical.

The mean serum cholesterol values of the hens while they were alive are also shown in Table I, as well as the degree of vascular sudanophilia. It may be noted that the group receiving coconut oil had the highest serum levels and those receiving corn oil the lowest. It seems likely that these differences, if significant, may be related to the dietary fat. Since production of eggs was variable in each hen and in each group, the data do not allow definite conclusions.[16] The mean gross vascular sudanophilia in the group receiving safflower oil would appear to be less extensive than that in the other dietary groups. No distinct differences in the degree of vascular sudanophilia could be detected among these latter groups, with the possible exception of somewhat less involvement in the hens fed olive oil. The incidence of grossly elevated sudanophilic lesions paralleled the degree of vascular sudanophilia.

The results of the analyses of the abdominal fat are shown in Table II together with analyses of the corresponding dietary fat. Typical chromatograms of the dietary and corresponding body fat are shown in Figures 1, 2 and 3. The similarity is such that any of the hens might be identified as to their dietary group by an inspection of the body fat analyses. In general, the analyses of fat from hens

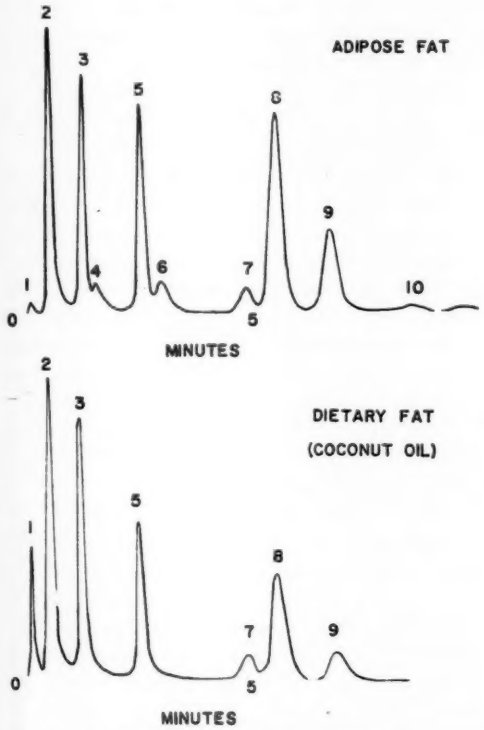

FIG. 1. A comparison of the chromatogram of coconut oil (bottom) and the adipose fat from a hen receiving 10 per cent coconut oil in the diet. The peaks are identified as the methyl esters of the following fatty acids: (1) capric, (2) lauric, (3) myristic, (4) myristoleic, (5) palmitic, (6) palmitoleic, (7) stearic, (8) oleic, (9) linoleic, (10) linolenic.

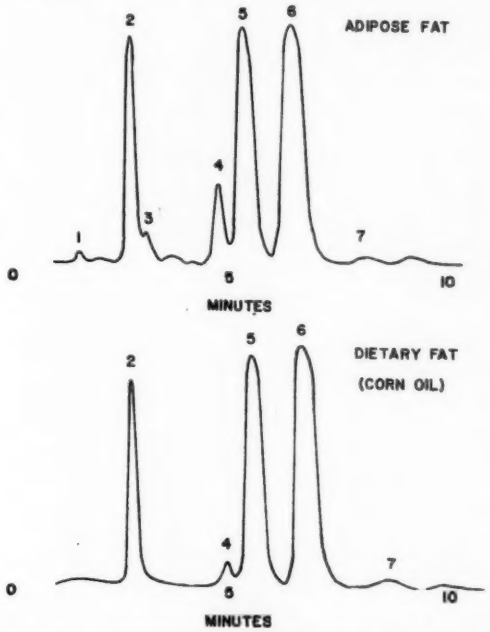

FIG. 2. Chromatograms obtained from corn oil and from the fat of hens fed 10 per cent corn oil. The peaks are identified as the methyl esters of the following fatty acids: (1) myristic, (2) palmitic, (3) palmitoleic, (4) stearic, (5) oleic, (6) linoleic, (7) linolenic. Several other minor constituents are present, particularly in the adipose fat.

within the same group did not vary more than ±5 per cent for the principal fatty acids although larger percentage variations were encountered for the fatty acids present in small amounts. Insufficient data are available to delineate the accuracy of the method for each fatty acid, particularly those present in small amounts, and the effects of variations in composition of the mixture upon the measurement of each constituent. The results are considered as semiquantitative only. The identity of a number of the minor constituents is inferred from the elution time since concentrates or pure samples were not available.

COMMENTS

The extent to which the fatty acid composition of the adipose tissue reflects the composition of the dietary fat is rather remarkable. Burr and Barnes[2] concluded, "It can hardly be said that any fat is typical of the species." On the other hand, it is clear that the animal is not entirely passive in the formation of tissue fat and some selection and modification of the dietary fatty acids is made. In the present studies the composition of corn oil and the fat from hens fed this oil were nearly identical in composition. One can deduce from the data from the other groups a tendency toward the formation of a fat of the corn oil type. Thus, the major constituents, palmitic, stearic, oleic and linoleic acid, when present in larger or smaller amounts in the dietary fat than occurred in the body fat of group 4 (fed corn oil), tend to be selectively retained or removed in the direction which would produce a fat of that composition. Thus, the body fat from group 1 (fed safflower oil) is lower in linoleic acid and higher in oleic acid than safflower oil itself, while the reverse relationship is seen in the group receiving olive oil. The extent to which the individual fatty acids are derived from the dietary source and from synthetic activities is, of course, unknown.

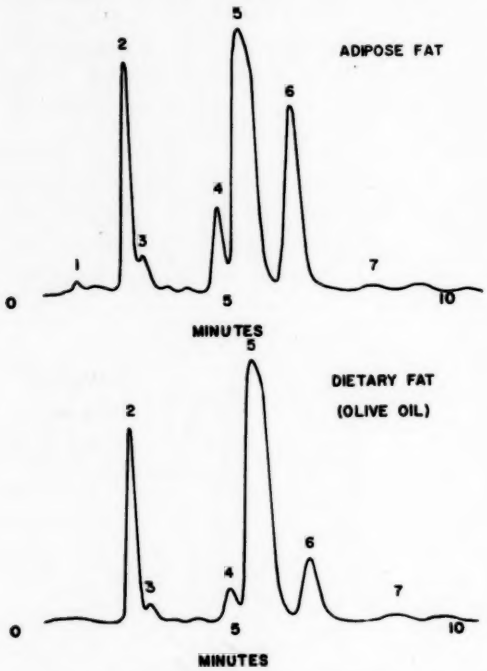

FIG. 3. Chromatograms obtained from olive oil and the fat of a hen fed 10 per cent olive oil. The peaks are identified as the methyl esters of the following fatty acids: (1) myristic, (2) palmitic, (3) palmitoleic, (4) stearic, (5) oleic, (6) linoleic, (7) linolenic. Several minor constituents are present in both samples.

The production and retention of some of the monounsaturated fatty acids, myristoleic and palmitoleic acids, is apparent in all groups. The dietary fat, with the exception of olive oil, contained very little of these fatty acids. It seems likely that these are derived in large part from the corresponding saturated acids since the content appears to be related partially to the intake of the fatty acids. This was particularly evident in the group fed coconut oil (group 2) which contained considerable myristic acid.

Undoubtedly, the changes in composition of body fat, as a result of changes in the diet, will be dependent upon both the composition of the dietary fat and the amount fed. The latter aspect has received relatively little study. The diets fed in this study contained 10 per cent fat, an amount higher than most common poultry rations, but relatively low in fat when compared to many diets consumed by man. The extent to which the kind and amount of dietary fat will be reflected in the tissue stores of various species, especially man, remains to be explored. It seems likely that analyses of the adipose tissue will reflect

the long term dietary pattern of the fatty acids consumed.

Those birds which received safflower oil in the diet had definitely less vascular sudanophilia than the other groups. It should be noted that none of the birds received cholesterol in the diet. The relationship of such differences in vascular sudanophilia to fat composition and serum cholesterol levels deserves further study.

SUMMARY

The fatty acid composition of the abdominal fat in hens was compared to the composition of the fat in the diet, the composition of the diets being otherwise identical. The proportion of fatty acids in the adipose tissue approximates that of the fat ingested. The findings, if applicable to man, suggest a method for estimating the dietary habits with regard to fat.

The hens consuming coconut oil had the highest serum cholesterol values. The amount of vascular sudanophilia was least in the group receiving safflower oil. Although it would appear that the kind of dietary fat influenced the serum cholesterol levels and the development of vascular sudanophilia, differences in production of eggs in this experiment limit the conclusions that can be drawn.

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Serum Levels of Vitamin B_{12} Following Intramuscular Injection of a Depot Preparation

S. D. Sobell, A.B.,* Aaron Arnold, Ph.D.† and Frederick Coulston, Ph.D.‡

In an earlier study[1] we presented clinical evidence showing that cobalamin administered intramuscularly in oil with aluminum monostearate was excreted into the urine over a prolonged period. This was in contrast with cobalamin administered in an aqueous medium. In the latter instance essentially all of the administered vitamin appeared to be recovered in the urine within twenty-four hours.

In view of the interesting effect of the oil-aluminum monostearate combination in prolonging the availability of the vitamin to the organism following injection, it appeared worthwhile to determine the extent to which the blood level of the vitamin might reflect the influence of the depot vehicle. Data on this point from studies with dogs are presented here.

METHODS

Control blood samples were taken from two groups of four dogs each apportioned as equally as possible on a weight basis. Following this, the vitamin in sterile water (Ducobee® "1000," 1,000 μg. vitamin B_{12} per ml.) or in sesame oil with 2 per cent aluminum monostearate (Ducobee Depot, 1,000 μg. vitamin B_{12} per ml.) was administered to each of the dogs intramuscularly (rectus femoris) in a dose of 15 μg. per kg. On a weight basis this amount approximated that given in the earlier clinical trial. Blood samples were then taken at one, two, three, four, seven, nine, eleven and fifteen days.

Blood samples, 8 to 10 ml. from the jugular vein, were taken from the dogs the morning before feeding so that they represent fasting samples. They were chilled for one hour to promote shrinking of the formed clot. They were then centrifuged for a half hour and the clear serum was taken for assay.

For estimation of the cobalamin content of serum, 1 to 2 ml. aliquots were diluted 1:5 with pH 4.6 acetate buffer following the procedure of Gaffney et al.,[2] steamed for a half hour, cooled to room temperature and centrifuged. An aliquot was diluted 1:2 with 0.33M Na_2HPO_4 to bring it to neutrality and then further diluted as needed for microbiologic assay by the U.S.P. procedure using L. leichmannii as the test organism. The extent of growth of the organism was based upon titration with 0.05N alkali of the lactic acid produced over a seventy-two–hour incubation period at 37°C. A comparison of the lactic acid production in the tubes containing the serum samples with the acid in the tubes containing the reference vitamin B_{12} permitted calculation of the vitamin B_{12} content of the serum samples.

To determine whether the depot preparation would exercise any untoward effect upon continued injection, it was administered twice weekly to two dogs (beagle) and two monkeys (rhesus) for a total of forty-six injections to each over a period of 170 days. Each of the four extremities were utilized in rotation. Hematologic studies were carried out on the animals before medication was started and at six-week intervals thereafter. At the end of the injection period, all of the animals were

From the Sterling-Winthrop Research Institute, Rensselaer, New York.

* Research Associate; † Director, Nutritional Research Laboratory; ‡ Director, Experimental Pathology and Toxicology Section.

TABLE I

Serum Vitamin B_{12} Levels in Two Groups of Four Dogs Each Following the Intramuscular Administration of the Vitamin in Water or in a Depot Oil and Aluminum Monostearate Preparation

Determination	Dog No.	Weight (kg.)	Serum Vitamin B_{12} Following Its Intramuscular Injection (mμg. per ml.)								
			Initial	Day							
				1	2	3	4	7	9	11	15
Aqueous Preparation											
	1	7.8	0.33	1.94	0.735	0.72	0.62	0 56	0.41	0.53	0.475
	2	7.0	0.33	1.69	0.675	0.51	0.64	0.42	0 39	0.39	0.32
	3	13.0	0.22	1.88	0.92	0.66	0.52	0.38	0.36	0.32	0.21
	4	12.6	0.33	1.79	0.72	0.64	0.54	0.36	0.33	0.34	0.23
Average			0.30	1.82	0.76	0.63	0.58	0.43	0.37	0.40	0.31
Standard error			±0.03	±0.06	±0 17	±0.04	±0.03	±0.04	±0.02	±0.05	±0.06
vs. initial			...	...	...	...	p = <0.001	p = 0.04	p = 0.07	p = 0.11	Not significant
Depot Preparation											
	5	5.8	0.36	2.50	1.97	1.75	1.02	0.765	0.71	0.785	0.54
	6	9.8	0.30	3 60	1.57	1.47	1.32	0.85	0.795	0.775	0.40
	7	11.6	0.33	2.50	1.62	1.10	0.95	0.46	0.34	0.35	0.22
	8	13.8	0.24	3.80	2.82	2.00	1.57	1.20	0.71	0.55	0.34
Average			0.31	3.10	2.00	1.58	1.22	0.82	0.64	0.62	0.38
Standard error			±0.02	±0.35	±0.29	±0.19	±0.14	±0.15	±0.10	±0.10	±0.07
vs. initial			...	...	...	...	...	p = 0.012	p = 0.014	p = 0.02	p = 0.4
vs. aqueous			...	...	...	p = 0.0014	p = 0.0025	p = 0.04	p = 0.03	p = 0.09	Not significant

examined grossly, and one of the monkeys was sacrificed for tissue section studies. The injection sites were imbedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

RESULTS

The results of the microbiologic analyses of the serum samples for vitamin B_{12} following the intramuscular injections of each of the preparations into the dogs are summarized in Table I. The average pretest serum vitamin B_{12} levels of 0.30 to 0.31 mμg. per ml. fall into the range of those noted for human beings by Chalmeras and Shinton[3] by assay with Euglena gracilis[4] and by Thompson and Hecht[5] by the procedure of Gaffney et al.[2]

From the average response to the administration of each of the vitamin B_{12} source preparations summarized in Table I and in Figure 1, it is seen that the dogs exhibited markedly elevated serum vitamin B_{12} levels. Those given the vitamin in an aqueous menstruum clearly had elevated levels through four days at which time their serum vitamin B_{12} values averaged 0.58 mμg. per ml. Since two or three of the four dogs of the group had serum vitamin B_{12} levels below 0.40 mμg. per ml. after four days, the significance of the average being above the pretest level may be questioned ($p = 0.04$ or higher).

It is interesting that the serum vitamin B_{12} levels of the four dogs given the vitamin as a depot preparation not only exhibited elevated levels over a longer period, between eleven and fifteen days, but also that the levels at-

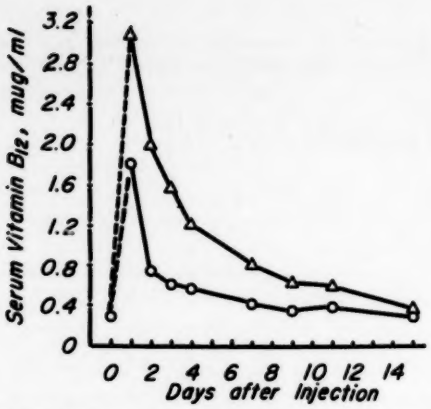

FIG. 1. Average serum vitamin B_{12} levels in dogs following the intramuscular injection of the vitamin in an aqueous (O—O) or depot (Δ—Δ) preparation.

tained averaged two to three times those of the dogs given the vitamin in an aqueous medium. This may reflect the fact noted earlier[1] that excess vitamin is quickly excreted following its administration in water. Release of the vitamin administered in oil containing aluminum monostearate took place more slowly as shown by prolonged urinary excretion. This is in accord with the above result which demonstrates elevated serum levels for a period of time about threefold longer than that following administration of the vitamin in the usual aqueous medium.

Of interest equal, perhaps, to the relative rates of excretion following the administration of the vitamin in an aqueous or depot preparation is the correlation between the data of this study and those of Buckwalter and Dickison.[6] Studying blood levels following the administration of procaine penicillin in oil and aluminum monostearate, these investigators noted that the vehicle supported elevated blood levels of the antibiotic for about twelve days. This is in striking agreement with the data given in Table I in which it is seen that the vitamin was clearly maintained at elevated levels for more than eleven (although less than fifteen) days.

With regard to the effects of the repeated injections in dogs and monkeys we may summarize briefly by noting that there was no apparent evidence of pain, redness or swelling at any time in any of the injection sites. No significant changes were observed in the total red and white blood cell counts, the differential counts, the hematocrit or in the hemoglobin concentration of the blood of the monkeys or the dogs. Microscopically, necrosis of muscle tissue was not seen although droplets of oil were observed in the connective tissue between the muscle bundles. Thus, it is seen that no adverse effects could be discerned and the preparation was completely tolerated as evidenced both by gross and microscopic examination of the sites of injection.

In conclusion, we may say that oil and aluminum monostearate serve as an effective depot vehicle for the intramuscular administration of vitamin B_{12} just as they do for procaine penicillin.

SUMMARY

Serum levels of vitamin B_{12} were determined in two groups of four dogs each following the intramuscular administration of vitamin B_{12} in water or in a depot oil and aluminum monostearate preparation.

Confirming earlier published observations based on the urinary excretion of the vitamin following its administration in each of these vehicles, the studies with the dogs showed that the vitamin administered in a depot form maintained elevated levels for a period about three times that following the administration of the vitamin in the usual aqueous medium.

It was noted, in addition, that the serum levels which followed the administration of the vitamin in the depot preparation were two to three times those which followed the administration of the vitamin in water.

Tolerance to injection of the preparation was established. No untoward blood or tissue effects were seen in dogs or monkeys following forty-six injections of the preparation over a 170-day period.

Accordingly, it is clear that administration of vitamin B_{12} in oil with aluminum monostearate results in prolonged and elevated serum levels of vitamin B_{12}.

ACKNOWLEDGMENT

The technical assistance of Mr. M. A. Rennie in these studies is gratefully acknowledged.

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Serum Lipids After Intravenous and Intragastric Fat Emulsion in Man

J. M. Iacono, PH.D.,* John F. Mueller, M.D.* and Lucille P. Wendel, B.S.

Considerable experience has been gained with the clinical use of fat emulsions for parenteral alimentation. Recently, a safe utilizable emusion for short term use has become available, and as more experience has gained, it has become possible to study the physiologic changes taking place in the removal of fat from the circulation. Initial observations by Mueller[1,2] have shown that there was great variation in the time required to clear fat from the blood stream, not only in different patients, but also in the same patient under varying circumstances. The mechanisms which regulate the disappearance of particulate lipid from blood are poorly understood, as are many of the other steps in lipid transport. It appeared to us, therefore, that fat emulsion might be a tool with which certain aspects of fat transport could be studied. The experiments described herein were designed to explore the role of the various serum lipid components in the removal of infused particulate fat.

An unpublished preliminary study was performed on nine normal volunteer subjects and nine hospitalized patients. The results served as a basis for the present studies in which the method of Van Eck, Peters and Man[3] for the separation of serum lipids into the "soluble and insoluble" fractions by high speed centrifugation was utilized. The present study lends further support to the theory that "soiuble" lipoprotein plays a role in the mechanisms involved in the transport of neutral fat.

METHODS AND PROCEDURE

Ten clinically healthy conscientious objectors between the ages of eighteen and twenty-one assigned to the metabolic Ward of the U. S. Army Medical Research and Nutrition Laboratory served as subjects (Table I). The men were divided into two groups. One group of six subjects was further subdivided into two groups of three men each. On the first day of the experiment one group received a single bottle of fat emulsion† intravenously and the other group received it intragastrically. On the following week, the route of administration was reversed. To the second group of four subjects was administered a daily intravenous infusion of fat emulsion for five days. The intravenous infusion of one bottle of fat emulsion lasted three and a half hours. When fat was administered intragastrically, it was delivered by nasogastric tube over a one-hour period. Blood samples were taken from the anticubital vein before and at four, eight, sixteen, twenty-four, thirty-six and forty-eight hours after the start of a single infusion. In the daily infusion experiment, blood samples were drawn before and at four, eight, sixteen and twenty-four hours after the start of the infusion on days one, three and five, and at twelve and twenty-four hours on day six. The cells were separated from the serum by centrifugation in a refrigerated International Centrifuge for twenty minutes at 2,400 r.p.m. Four ml. of the original serum were taken for analysis and the remainder, usually 7 to 8 ml., was transferred to lustroid tubes and spun in a

From the U. S. Army Medical Research and Nutrition Laboratory, Denver, Colorado

* Present address: Department of Internal Medicine, University of Cincinnati, Cincinnati General Hospital, Cincinnati, Ohio.

† Kindly supplied by the Upjohn Co., Kalamazoo, Michigan. Lipomul® (I.V.) consists of 15 per cent cottonseed oil, 1.2 per cent soybean phosphatide, and 0.3 per cent Pluronic (F-68) in 4 per cent dextrose solution. One bottle contains 600 ml. of fluid.

TABLE I
Height and Body Weight of Subjects

Subject No.	Height (cm.)	Weight (kg.)
Daily Infusion Study		
1	169	64.1
2	168	72.3
3	178	65.9
4	171	66.9
Single Infusion Study		
5	176	72.7
6	173	63.6
7	176	92.3
8	179	70.3
9	176	85.0
10	173	61.8

TABLE II
Composition of Diets

Constituents	gm.	%
*Control Diet**		
Protein (N × 6.25)	130	19.8
Fat	111	16.9
Carbohydrate	395	60.3
Ash	19	2.9
Low Fat Diet†		
Protein (N × 6.25)	132	18.7
Fat	32	4.5
Carbohydrate	522	74.0
Ash	19	2.9

* Metabolizable energy, 3,100 calories.
† Metabolizable energy, 2,900 calories.

Servall Super-Centrifuge at 30,000 × G for 45 minutes in a cold room (4°C.). About 5 ml. of the clear subnatant serum were removed from the bottom of each tube by a previously described method.[4] Great care was taken to avoid disturbance of the cream layer as the clear subnatant was withdrawn. It was found, using this procedure, that the subnatant serum was cleared of practically all chylomicrons after forty-five minutes of centrifugation when examined by dark field microscopy and that the measurable optical density of the subnatant serum compared to distilled water at 700 mμ was 0.08 or less in a Coleman Jr. spectrophotometer, indicating that there were few, if any, chylomicrons present. For this method to have validity, the lipids must be uniformly distributed in the clear subnatant. Van Eck, Peters and Man[3] have shown this to be the the case except in heavily lipemic serum associated with lipid disorders.

Lipid Analyses: We have found that a single extract of serum with acetone: ethanol (1:1 v/v) which is generally used only for extraction of cholesterol was also suitable for extracting the neutral fats and phospholipids since their recoveries were similar to those obtained when using Bloor's mixture of alcohol: ether (3:1 v/v). Therefore, a single extract of whole serum and of subnatant serum was prepared for all lipid analyses by diluting exactly 4 ml. of serum with acetone: alcohol to 100 ml. in a volumetric flask. Before diluting to final volume, the serum was pipetted into the volumetric flask containing about 25 ml. of the solvent and heated to boiling in a water bath. The preparation was filtered under watchglasses into 100 ml. glass stoppered cylinders to remove the precipitated protein, and aliquots of this extract were taken for lipid analysis. Total fatty acids,[5] cholesterol[6] and lipid phosphorus[7] were measured. Total fatty acids and neutral fatty acids are expressed as mEq./L. Lipid phosphorus and cholesterol are expressed as mg. per cent. The calculations for neutral fatty acids (triglyceride fatty acids) and phospholipid fatty acids were based on the method of Albrink, Man and Peters.[4] We prefer the term neutral fatty acids since by this method monoglycerides, diglycerides and non-esterified fatty acids are included in this compartment. In the experiment in which non-esterified fatty acids were determined, the method of Davis as modified by Grossman, Palm, Becker and Moeller[8] was used. It should be pointed out that values for esterified cholesterol and netural fatty acids are derived values subject to the combined errors of two or more analyses and that the values reported for those components will reflect the sum total errors of the methods. This is particularly true for

TABLE III

Changes in Whole Serum and Subnatant Serum Lipids After a Single Intravenous and Intragastric Infusion of Fat Emulsion in Healthy Young Men

	Subject No.	Hour																						
		0	4	8	16	24	36	48	0	4	8	16	24	36	48	0	4	8	16	24	36	48
		Total Fatty Acids (mEq./L.)							*Neutral Fatty Acids (mEq./L.)*							*Lipid Phosphorous (mg. %)*						
Intravenous administration																								
Whole serum	5	10.0	58.9	24.6	8.2	8.8	12.0	12.4	3.4	50.0	17.2	...	2.9	5.5	...	7.3	11.4	9.0	7.2	6.3	7.6	8.1
	6	13.8	71.3	59.4	10.7	10.6	17.5	16.2	...	58.3	48.4	1.8	2.2	8.6	6.8	...	15.1	11.7	9.4	8.1	9.1	10.1
	7	10.9	37.1	19.2	9.4	9.0	14.5	11.3	2.0	29.1	9.9	2.5	2.2	6.0	3.5	8.8	8.0	10.0	6.6	6.8	9.2	8.2
	8	5.8	34.0	16.8	4.8	7.0	8.5	10.0	...	27.3	10.6	0.6	2.1	3.1	3.8	5.2	8.4	6.7	4.1	5.1	6.3	5.9
	9	12.2	52.8	26.2	8.5	11.3	13.8	16.2	3.7	42.2	18.1	...	3.7	4.9	8.1	8.9	12.2	7.8	6.9	7.4	9.6	8.6
	10	11.8	65.9	35.4	7.8	9.4	15.8	14.5	3.1	55.3	26.4	1.0	...	7.6	6.3	8.5	12.5	10.0	6.6	...	9.4	8.9
Mean ± s.d.		10.7	53.3	30.3	8.2	9.4	13.7	13.4	3.1	43.7	21.8	1.5	2.6	6.0	5.7	7.8	11.3	9.2	6.8	6.7	8.5	8.3
		±2.7	±15.1	±15.6	±2.0	±1.5	±3.1	±2.6	±0.7	±13.2	±14.4	±0.9	±0.2	±2.0	±2.0	±1.6	±2.7	±1.8	±1.7	±1.1	±1.3	±1.4
Subnatant serum	5	9.7	15.1	15.9	8.5	8.2	10.7	11.6	2.8	...	8.5	1.7	2.3	4.1	4.4	7.3	9.4	9.0	7.6	6.4	7.5	8.1
	6	13.3	16.6	20.0	10.9	10.3	12.9	14.5	...	6.6	10.7	2.4	2.0	4.5	5.7	...	10.7	10.2	8.0	7.8	8.0	8.5
	7	11.5	...	17.8	9.3	9.8	13.7	11.1	3.8	...	9.7	...	2.9	5.5	3.2	7.4	...	9.0	7.2	7.1	9.3	8.6
	8	7.4	13.1	12.6	4.8	6.7	7.7	8.9	...	...	...	...	...	...	...	6.3	7.1	6.0	2.4	5.4	3.5	4.3
	9	...	...	16.9	8.1	10.3	13.7	14.4	...	...	8.6	...	3.5	5.0	7.0	...	9.0	8.3	7.6	6.5	9.1	7.4
	10	10.8	15.7	19.6	7.7	8.8	14.6	13.7	2.6	7.4	12.0	1.7	2.4	8.3	6.0	8.1	8.9	8.2	5.5	6.4	5.7	7.7
Mean ± s.d.		10.5	15.1	17.1	8.2	9.0	12.2	12.4	3.1	7.0	9.9	1.9	2.6	5.5	5.2	7.3	9.0	8.4	6.4	6.6	7.2	7.4
		±2.2	±1.5	±2.7	±2.0	±1.4	±2.6	±2.2	±0.6	±0.2	±1.5	±0.1	±0.2	±1.7	±1.5	±0.7	±1.3	±1.4	±2.2	±0.8	±2.2	±1.6
Intragastric administration																								
Whole serum	5	10.2	16.8	9.3	8.5	9.4	11.4	11.6	2.2	7.6	1.8	1.4	2.0	4.8	4.3	8.2	9.8	8.3	7.3	7.4	7.2	7.7
	6	11.0	13.0	29.7	10.2	9.8	11.3	13.1	1.7	2.9	20.0	1.1	1.2	...	5.4	9.5	10.4	10.3	9.8	8.4	8.4	7.6
	7	13.2	17.6	31.6	12.4	13.6	15.8	16.0	3.2	7.7	22.6	4.6	...	7.6	7.3	10.1	10.0	8.8	7.6	8.8	8.7	9.0
	8	9.1	11.9	7.5	6.4	7.4	9.9	7.2	1.5	5.8	2.1	0.8	1.8	4.2	2.2	9.0	7.1	6.0	6.1	6.4	6.6	5.9
	9	11.7	15.1	15.2	10.2	10.2	13.4	14.8	3.7	7.0	6.4	1.8	2.9	5.4	6.5	7.8	7.7	8.2	8.4	6.8	8.2	8.4
	10	13.5	17.4	10.6	10.4	11.4	14.9	13.8	4.8	8.4	2.5	1.8	3.8	7.0	6.1	8.9	9.2	8.3	9.0	7.6	8.2	8.2
Mean ± s.d.		11.5	15.3	17.3	9.7	10.3	12.8	12.8	2.9	6.6	9.2	1.9	2.3	5.8	5.3	8.9	9.0	8.3	8.0	7.6	7.9	7.8
		±1.7	±2.4	±10.6	±2.0	±2.1	±2.3	±3.1	±1.3	±2.0	±9.5	±1.4	±1.0	±1.4	±1.8	±0.8	±1.3	±1.4	±1.3	±0.8	±0.8	±1.1
Subnatant serum	5	10.2	13.4	9.4	7.6	9.8	10.6	11.2	3.1	5.5	2.4	1.1	4.2	3.4	5.4	8.3	7.9	7.8	6.8	4.2	7.9	5.3
	6	10.4	11.7	12.0	9.8	10.0	11.2	13.1	2.0	2.7	...	2.0	1.4	4.8	4.8	9.0	9.1	8.5	7.7	8.3	5.7	8.4
	7	12.6	13.8	12.2	10.8	13.0	15.0	15.8	3.5	4.7	3.1	4.4	...	8.3	7.6	8.8	8.8	9.9	6.2	8.1	5.9	7.9
	8	8.6	9.0	7.1	5.3	7.4	7.4	7.6	2.4	2.9	0.8	0.0	1.8	2.4	2.7	7.1	7.1	7.4	6.0	6.2	5.9	5.6
	9	11.1	13.2	12.8	9.6	9.5	12.2	13.6	3.1	4.9	3.6	1.7	1.7	4.3	5.8	8.1	8.5	9.8	7.9	7.4	5.8	7.6
	10	12.6	14.5	10.3	10.6	11.2	13.9	14.6	4.2	5.7	2.2	2.3	3.8	7.4	7.1	7.9	8.5	8.5	8.4	7.1	5.9	7.9
Mean ± s.d.		10.9	12.6	10.6	9.0	10.2	11.7	12.6	3.1	4.4	2.4	1.9	2.6	5.1	5.7	8.2	8.3	8.6	7.2	6.9	6.2	7.1
		±1.5	±2.0	±2.2	±2.1	±1.9	±2.7	±2.9	±0.8	±1.3	±1.0	±1.4	±1.3	±2.3	±1.8	±0.7	±0.7	±0.9	±1.0	±1.5	±0.8	±1.3

TABLE III *Continued*

	Subject No.	Hour																						
		0	4	8	16	24	36	48	0	4	8	16	24	36	48	0	4	8	16	24	36	48
		Total Cholesterol (mg. %)							*Free Cholesterol (mg. %)*							*Esterified Cholesterol (mg. %)*						
Intravenous administration																								
Whole serum	5	134	142	139	117	125	122	...	44	55	56	39	37	42	47	90	87	83	78	88	80	...
	6	240	236	232	189	192	200	201	65	74	73	56	58	62	64	174	162	160	133	134	138	137
	7	196	186	191	158	154	169	165	49	55	57	41	43	48	45	146	132	134	117	111	121	120
	8	...	...	...	...	...	...	...	22	50	44	25	33	33	32	...	...	...	...	...	...	...
	9	171	210	203	...	179	185	172	44	74	64	42	50	57	53	126	136	137	...	129	128	119
	10	183	192	184	152	152	155	168	43	63	62	40	41	50	50	143	129	122	112	110	105	119
Mean ± s.d.		185	193	190	154	160	166	176	45	62	59	40	44	49	48	136	129	127	110	114	114	124
		±38	±35	±34	±30	±26	±30	±17	±14	±10	±10	±10	±9	±11	±11	±31	±27	±28	±23	±18	±23	±9
Subnatant serum	5	144	129	124	135	123	125	137	42	..	46	45	37	41	40	102	...	78	90	86	85	97
	6	232	206	194	205	203	205	212	64	59	63	56	55	59	61	168	147	131	149	147	146	150
	7	180	...	165	145	150	155	158	48	..	53	..	42	46	44	131	...	112	87	108	109	113
	8	...	...	...	...	...	...	...	34	34	35	26	29	29	29	...	...	...	...	...	...	...
	9	...	183	189	...	164	186	168	..	52	54	42	46	56	49	...	131	136	...	118	130	119
	10	184	162	162	146	144	162	171	50	43	50	36	40	48	50	134	119	112	110	104	114	122
Mean ± s.d.		185	170	167	158	157	167	169	48	47	50	41	42	46	46	123	132	114	109	101	117	120
		±36	±33	±28	±32	±30	±31	±27	±11	±11	±9	±11	±9	±11	±11	±33	±14	±23	±29	±22	±23	±19
Intragastric administration																								
Whole serum	5	166	178	156	147	164	132	147	41	45	50	37	45	41	40	125	133	106	111	119	91	107
	6	198	220	195	184	199	...	184	54	63	52	54	55	56	55	145	157	143	131	144	...	129
	7	212	226	202	184	...	183	195	55	69	53	56	..	63	63	157	157	149	129	...	120	132
	8	125	109	106	106	101	100	84	35	34	31	26	28	29	24	90	75	75	80	74	71	60
	9	180	188	202	179	163	170	178	46	49	47	42	41	44	47	134	139	155	137	122	126	131
	10	186	192	172	171	164	161	153	49	52	44	42	42	42	39	137	141	128	129	122	119	114
Mean ± s.d.		178	186	172	162	158	149	157	47	52	46	43	42	46	45	131	134	126	119	116	106	135
		±30	±42	±37	±31	±35	±33	±40	±8	±12	±8	±11	±10	±12	±14	±23	±31	±31	±21	±26	±22	±28
Subnatant serum	5	131	176	150	137	161	138	141	43	50	57	39	40	37	38	88	126	94	98	121	101	103
	6	180	216	175	180	199	...	186	56	74	..	51	53	50	54	124	142	...	129	147	...	132
	7	220	221	189	166	...	175	191	65	68	48	55	..	50	51	155	153	141	112	...	125	140
	8	114	104	106	97	104	85	91	34	31	30	25	28	24	27	79	73	76	72	75	62	65
	9	173	180	180	169	173	173	172	44	47	46	42	20	39	41	129	132	134	127	134	134	131
	10	193	195	165	173	169	158	155	46	48	42	41	42	41	43	147	147	123	132	127	118	112
Mean ± s.d.		168	182	161	153	161	146	156	48	53	45	42	40	40	42	121	104	114	111	121	108	114
		±40	±42	±30	±31	±35	±37	±37	±11	±16	±10	±10	±9	±10	±10	±31	±58	±28	±23	±27	±28	±28

the values for neutral fatty acids. However, the methods used in these studies are the classic methods for serum lipids and have been widely accepted. In some instances, because of technical problems, individual data are missing, thereby affecting the validity of the mean values shown on the tables and graphs. In all instances, all of the data obtained are reported in the tables for the scrutiny of the reader.

Dietary: All subjects were on diets of natural foodstuffs, and the caloric values of the control and low fat diets were approximately the same. The composition of the diets is shown in Table II. In the low fat diet, carbohydrate was substituted in isocaloric amounts for butter. The caloric value of Lipomul, about 950 calories, was supplementary to the diet. As a prefeeding period, the control diet was consumed by all subjects for five days. Those receiving a single bottle of Lipomul were fed the low fat diet for two days following the administration of the fat emulsion. The control diet was reinstituted again until they received the second bottle of fat one week later. Those subjects receiving the daily infusions were changed from the control diet to the low fat diet on the day of the first infusion and remained on the low fat diet for the rest of the experiment.

RESULTS

Single Administration: The intravenous and intragastric administration of Lipomul resulted in changes in serum lipids that were somewhat different (Table III). The data for whole serum neutral fatty acids in the subjects receiving single intravenous infusions showed an average increase of 40.6 mEq. per L. over the control value. One bottle of Lipomul contains about 330 mEq. of neutral fatty acids based on an average fatty acid molecular weight of 260. Assuming a plasma volume of 3 L., 121.8 mEq. of neutral fatty acids remained in the serum at the end of infusion. Therefore, during the infusion period 208.2 mEq. or about 63 per cent of the infused fat was removed from the blood stream. On an average rate basis, about 16.5 mEq. per L. per hour were removed during the infusion.

TABLE IV

Changes in Whole Serum Non-esterified Fatty Acids Following a Single Intravenous Infusion of Lipomul in Six Healthy Human Subjects*

Sex and Age (yr.)	0 hr.	4 hr.	6 hr.	8 hr.
Female, 23	0.38	1.18	0.56	0.42
Female, 38	0.25	1.52	0.75	0.43
Male, 25	0.32	1.05	0.53	0.44
Female, 36	0.22	1.03	0.64	0.62
Male, 29	0.30	1.42	0.88	0.52
Male, 22	0.34	1.82	1.17	0.68
Mean	0.30	1.34	0.76	0.52

NOTE: All figures shown are in mEq./L.

* Subjects were fasted overnight for at least twelve hours.

In the four-hour postinfusion period a total of 65.7 mEq. of fat were cleared, and the remainder disappeared between the eighth and sixteenth hours. Values for subnatant neutral fatty acids reached a maximum eight hours after the start of the intravenous infusion and this increase was usually three to eight times greater than the fasting value.

Whole serum neutral fatty acid values following nasogastric feeding of the fat emulsion suggest in three of six cases that the ingested lipid cleared the blood stream within eight hours since the levels of neutral fatty acids returned to preinfusion values by that time. Whole serum neutral fatty acids, of course, did not rise to the levels observed after intravenous infusion and the time at which the neutral fatty acid peaked was variable. Much smaller increases in neutral fatty acids were observed in the subnatant serum of subjects receiving the emulsified fat by nasogastric tube.

Changes in whole serum lipid phosphorus generally paralleled neutral fatty acids when a single bottle of Lipomul was administered intravenously. The maximum increase in whole serum lipid phosphorus occurred at the end of the infusion and could be accounted for by the phosphatide in the emulsion. Subnatant serum lipid phosphorus also rose during the infusion and thereafter fell below the fasting level. Subjects receiving the emulsion intra-

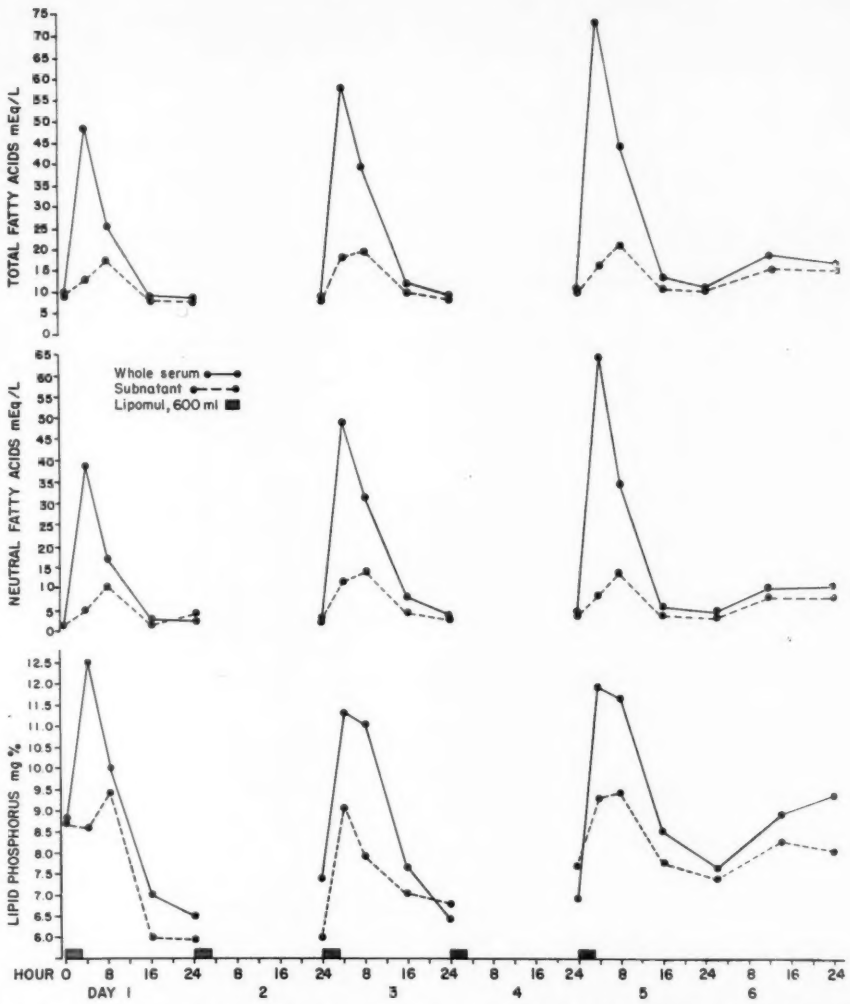
TOTAL FATTY ACIDS mEq/L
NEUTRAL FATTY ACIDS mEq/L
LIPID PHOSPHORUS mg %
Whole serum
Subnatant
Lipomul, 600 ml
HOUR 0 8 16 24 8 16 24 8 16 24 8 16 24 8 16 24 8 16 24
DAY 1 2 3 4 5 6

FIG. 1. Average values for total fatty acids, neutral fatty acids and lipid phosphorus in whole serum and subnatant serum of four healthy young adults on days one, three and five following daily intravenous infusion of fat emulsion.

gastrically showed little change in whole serum and subnatant lipid phosphorus.

An initial rise in whole serum free cholesterol occurred following the oral and intravenous feeding of Lipomul. Small amounts of cholesterol-like substances (Liebermann-Burchard positive plant sterols) are present in Lipomul, but an increase in serum due to these plant sterols should have been only 3 to 4 mg. per cent, whereas greater increases were recorded. Average values for serum cholesterol decreased 10 to 15 per cent in both groups after twenty-four hours. Most of the decrease occurred in the esterified cholesterol fraction. Within forty-eight hours after the administration of Lipomul, either intravenously or intragastrically, all serum lipids returned to preinfusion levels.

In a separate study, non-esterified fatty acids were measured to evaluate their contribution fatty acids of serum after intravenous Lipomul (Table IV). The data from six

TABLE V

Changes in Whole Serum and Subnatant Serum Lipids After Daily Infusion of Fat Emulsion in Healthy Young Men

	Subject No.	Hour																						
		0	4	8	16	24	36	48	0	4	8	16	24	36	48	0	4	8	16	24	36	48
		Total Fatty Acids (mEq./L.)							*Neutral Fatty Acids (mEq./L.)*							*Lipid Phosphorus (mg. %)*						
Day one																						
Whole serum	1	10.6	38.0	17.0	8.6	8.4	...	...	1.4	27.6	8.1	1.2	1.8	...	...	9.5	12.2	10.6	7.3	6.3	. .	...
	2	10.0	43.0	22.0	9.2	8.2	...	...	2.2	33.7	13.8	2.1	2.1	...	...	7.3	10.3	8.7	6.6	6.4	...	...
	3	9.4	58.5	32.4	10.2	10.0	...	...	0.3	46.1	22.3	2.9	2.7	...	...	9.6	15.5	10.9	7.9	7.3	...	...
	4	10.7	56.0	33.6	9.3	9.2	...	...	1.4	45.3	24.4	3.1	2.8	...	...	8.7	12.0	10.0	6.0	6.1	...	...
Mean ± s.d.		10.2	48.9	26.2	9.3	9.0	...	...	1.3	38.2	17.2	2.3	2.4	...	...	8.8	12.5	10.0	7.0	6.5	...	...
		±0.6	±9.9	±8.1	±0.7	±0.8			±0.8	±9.0	±7.6	±1.0	±0.5			±1.1	±2.2	±1.0	±0.8	±0.5		
Subnatant serum	1	10.6	15.2	16.1	8.2	8.1	...	...	1.3	7.8	8.4	0.9	1.6	...	...	9.6	7.9	8.9	7.5	6.5	...	...
	2	8.8	11.0	17.6	6.7	8.0	...	...	0.8	2.5	10.3	1.2	2.7	...	...	8.3	9.4	8.5	5.7	5.5	...	...
	3	9.2	13.6	19.6	9.7	9.2	...	...	1.1	4.9	10.2	2.7	3.9	...	...	9 1	9.3	11.1	7.1	4.7	...	...
	4	10.3	12.2	18.0	8.6	8.7	...	...	2.2	4.2	10.2	3.6	2.1	...	...	7.7	7.9	8.9	3.8	6.9	...	...
Mean ± s.d.		9.7	13.0	17.8	8.3	8.5	...	...	1.4	4.9	10.0	2.1	2.6	...	...	8.7	8.6	9.4	6.0	5.9	...	...
		±0.9	±1.8	±1.4	±1.2	±0.6			±0.6	±2.2	±0.9	±1.3	±1.0			±0.8	±0.8	±1.2	±1.6	±1.0		
Day three																						
Whole serum	1	8.7	57.2	36.9	10.2	9.2	...	...	1.3	48.4	27.1	2.9	3.0	...	...	7.9	11.1	11.9	8.1	7.3	...	...
	2	8.0	43.6	29.3	9.4	9.8	...	...	2.1	35.5	21.2	4.1	5.1	...	...	6.6	10.3	10.4	5.9	5.4	...	...
	3	9.7	67.8	52.3	19.8	9.6	...	...	2.5	57.5	42.8	11.7	3.2	...	...	7.6	12.7	11.3	10.1	7.3	...	...
	4	10.0	65.8	43.8	10.8	10.2	...	...	...	56.7	34.4	4.4	4.5	...	...	...	11.0	10.9	6.9	5.8	...	...
Mean ± s.d.		9.1	58.6	40.6	12.6	9.7	...	...	2.0	49.6	31.4	5.8	4.0	...	...	7.4	11.3	11.1	7.7	6.4	...	...
		±0.9	±11.0	±9.8	±4.9	±0.4			±0.6	±10.3	±9.3	±4.0	±1.0			±0.6	±1.0	±0.7	±1.8	±1.0		
Subnatant serum	1	9.1	17.8	19.9	9.2	9.0	...	...	3.3	10.8	13.4	2.4	2.8	...	...	5.0	8.4	6.9	7.3	6.2	...	...
	2	8.0	18.0	20.6	8.3	9.0	...	...	2.4	11.5	15.1	1.9	3.3	...	...	5.8	7.8	7.3	7.6	6.3	...	...
	3	9.8	19.8	20.8	16.2	9.1	...	...	3.7	11.6	13.2	9.4	2.0	...	...	5.8	10.4	9.2	7.1	7.9	...	...
	4	8.9	19.9	20.4	9.8	9.3	...	...	1.9	11.9	13.1	3.5	2.7	...	...	7.5	9.6	8.2	6.5	7.0	...	...
Mean ± s.d.		9.0	18.9	20.4	10.9	9.1	...	...	2.8	11.4	13.7	4.3	2.7	...	...	6.0	9.1	7.9	7.1	6.8	...	...
		±0.7	±1.1	±0.4	±3.6	±0.1			±0.8	±0.4	±0.9	±3.5	±0.5			±1.1	±1.2	±1.1	±0.5	±0.8		
Day five																						
Whole serum	1	10.2	85.4	39.1	9.1	10.1	17.1	15.6	3.6	76.6	29.7	2.6	3.5	9.8	7.2	6.7	10.9	11.5	7.3	7.3	8.0	9.4
	2	11.3	61.3	37.3	10.4	12.5	19.3	18.4	5.0	52.3	28.1	3.9	5.9	11.7	10.2	7.3	11.8	11.8	8.0	8.0	8.9	9.6
	3	10.9	72.4	47.3	15.7	11.6	18.2	14.3	4.7	63.0	37.5	8.2	4.4	9.8	5.8	6.3	12.1	11.9	8.9	7.9	9.7	9.3
	4	13.3	75.7	53.7	17.0	12.4	18.5	19.6	6.2	66.0	43.6	8.4	5.6	10.3	10.7	7.5	12.7	11.6	10.4	7.4	9.1	9 7
Mean ± s.d.		11.4	73.7	44.4	13.0	11.6	18.3	17.0	4.9	64.5	34.7	5.8	4.9	10.4	8.5	6.9	11.9	11.7	8.6	7.6	8.9	9.5
		±1.3	±9.9	±7.6	±3.9	±1.1	±0.9	±2.4	±1.1	±10.0	±7.2	±3.0	±1.1	±0.9	±2.4	±0.5	±0.8	±0.2	±1.3	±0.4	±0.7	±0.2
Subnatant serum	1	9.5	15.2	20.2	9.1	8.9	13.5	14.2	3.6	7.7	11.7	2.0	2.2	5.9	6.8	7.4	9.2	9.9	7.8	7.3	8.2	8.5
	2	10.8	16.0	23.5	9.2	11.5	16.4	17.6	4.8	8.9	16.1	2.5	5.0	9.0	10.3	7.4	9.0	8.9	7.5	7.3	9.1	8.1
	3	10.4	16.6	20.2	12.4	10.7	15.1	13.4	3.8	9.1	12.8	5.3	4.0	9.1	6.3	8.3	9.8	9.0	8.0	7.7	7.1	7.5
	4	11.8	19.6	22.7	14.4	11.4	16.3	...	4.3	11.9	14.3	7.1	4.6	8.1	...	7.9	8.8	9.9	8.0	7.4	8.8	8.5
Mean ± s.d.		10.6	16.8	21.6	11.3	10.6	15.3	15.1	4.1	9.4	13.7	4.2	4.0	8.0	7.8	7.7	9.3	9.4	7.8	7.4	8.3	8.1
		±1.0	±1.9	±1.8	±2.6	±1.2	±1.4	±9.5	±0.5	±2.8	±1.9	±2.4	±1.2	±1.5	±2.2	±0.4	±0.4	±0.6	±0.2	±0.2	±0.9	±0.5

TABLE V *Continued*

	Subject No.	Hour																						
		0	4	8	16	24	36	48	0	4	8	16	24	36	48	0	4	8	16	24	36	48
		Total Cholesterol (mg. %)							*Free Cholesterol (mg. %)*							*Esterified Cholesterol (mg. %)*						
Day one																						
Whole serum	1	190	181	186	166	155	...	...	47	51	60	45	43	..	..	143	130	126	121	112	...	...
	2	180	191	184	175	132	...	...	42	62	61	51	41	..	..	138	129	122	124	91	...	...
	3	180	193	214	154	167	...	...	47	62	70	48	47	..	..	134	132	144	105	120	...	...
	4	186	212	200	148	158	...	...	49	67	70	42	48	..	..	137	145	130	106	111	...	...
Mean ± s.d.		184	194	196	161	153	...	...	46	60	65	47	45	..	..	138	134	131	114	108	...	...
		±5	±13	±14	±12	±15			±3	±4	±5	±4	±3			±4	±8	±16	±10	±12		
Subnatant serum	1	192	154	170	155	145	...	...	50	44	72	41	40	..	..	142	110	98	114	104	...	...
	2	168	169	156	124	122	...	...	46	50	65	39	40	..	..	122	119	92	85	82	...	...
	3	162	176	172	157	147	...	...	51	50	59	48	47	..	..	111	126	113	109	100	...	...
	4	192	179	164	148	141	...	...	54	47	62	42	42	..	..	138	132	102	106	99	...	...
Mean ± s.d.		178	170	166	146	139	...	...	50	48	64	42	42	..	..	128	122	101	103	96	...	...
		±16	±11	±7	±15	±12			±3	±3	±6	±6	±3			±14	±9	±9	±13	±10		
Day three																						
Whole serum	1	151	142	178	141	...	...	...	43	50	69	42	33	..	..	108	91	109	100	...	...	...
	2	117	134	144	110	98	...	...	37	52	63	38	37	..	..	80	82	81	72	62	...	...
	3	152	160	183	150	124	...	...	45	59	70	62	41	..	..	107	103	112	88	83	...	...
	4	151	165	187	138	133	...	...	43	60	70	45	43	..	..	107	105	117	94	90	...	...
Mean ± s.d.		143	151	173	135	118	...	...	42	55	68	47	40	..	..	101	95	105	88	78	...	...
		±17	±15	±20	±17	±18			±4	±5	±3	±11	±3			±14	±11	±16	±12	±15		
Subnatant serum	1	153	114	145	139	...	...	...	42	34	49	40	40	..	..	111	81	95	98	...	...	...
	2	120	113	102	114	116	...	...	35	36	51	35	39	..	..	85	77	51	78	77	...	...
	3	150	124	140	158	133	...	...	44	40	51	56	36	..	..	106	84	89	102	97	...	...
	4	145	133	148	140	138	...	...	42	38	52	44	40	..	..	103	94	97	96	97	...	...
Mean ± s.d.		142	121	134	137	129	...	...	41	37	51	44	39	..	..	101	84	83	94	91	...	...
		±15	±9	±21	±18	±11			±4	±3	±1	±9	±2			±12	±8	±22	±10	±12		
Day five																						
Whole serum	1	146	152	175	129	131	148	166	43	57	69	40	40	49	52	103	95	106	89	91	100	114
	2	119	140	162	115	120	144	151	42	59	71	43	45	52	52	77	81	91	72	76	93	99
	3	143	148	185	146	146	162	174	46	57	73	53	48	55	53	97	91	112	92	98	107	121
	4	153	177	209	151	144	174	189	46	66	82	51	47	61	60	107	110	128	100	96	113	128
Mean ± s.d.		140	154	183	135	135	157	170	44	60	74	47	45	54	54	96	94	109	88	90	103	116
		±15	±16	±20	±16	±12	±13	±16	±2	±4	±6	±6	±4	±5	±4	±13	±12	±15	±12	±10	±9	±12
Subnatant serum	1	100	121	143	133	129	141	138	39	36	48	35	34	42	42	61	85	95	97	94	99	97
	2	105	110	135	120	128	123	142	38	35	50	36	42	44	44	67	72	85	84	85	80	99
	3	110	110	133	135	124	117	156	41	39	50	42	41	44	48	69	71	83	93	84	74	108
	4	150	146	156	144	140	168	160	38	44	54	42	42	48	53	112	102	102	102	99	120	107
Mean ± s.d.		116	122	142	133	130	137	149	39	39	50	39	40	44	46	78	82	92	94	99	93	103
		±23	±17	±10	±10	±7	±23	±10	±2	±3	±2	±4	±4	±2	±5	±24	±15	±9	±8	±7	±21	±6

healthy subjects fasted overnight indicated that non-esterified fatty acids rose to an average value of 1.34 mEq./L. at four hours and thereafter decreased, but did not reach the preinfusion value by eight hours.

Daily Infusions: The changes observed on the first day of the multiple infusion experiment were generally noted on days three and five. A progressive rise in neutral fatty acids in whole serum occurred at the end of successive infusions. The average values for the four subjects on days one, three and five were 38.2, 49.6 and 64.5 mEq. per L., respectively, indicating a progressive decrease in the rate of clearing of the infused fat. At the same time, an increase in the average neutral fatty acid values of fasting serum samples of 1.3, 2.0 and 4.9 mEq. per L. for days one, three and five was noted. It would appear from the aforementioned that an inverse relationship exists between the fasting neutral fatty acid levels in serum and the rate of clearing of infused fat. The increase in subnatant neutral fatty acids observed with a single intravenous infusion of fat was also observed in each instance of the daily infusion study (Fig. 1, Table V).

Changes in lipid phosphorus during day one were analogous to changes in neutral fatty acids, however, on days three and five, phospholipids did not decrease from the fourth to the eighth hour, whereas neutral fatty acids, decreased considerably in the same time interval. The maximum drop in fasting phospholipids occurred at the end of the first day when the average value in serum decreased from 8.8 to 6.4 mg. per cent, representing a 27 per cent decline (Fig. 1, Table V).

On the first day the serum cholesterol behaved much as it had in the previous experiment. By the third day, greater initial rises followed by lesser total decreases were noted. On the fifth day the pattern had changed considerably from the first day. At this time a rise in both whole serum and subnatant was followed by a fall to preinfusion levels. However, the average serum cholesterol decreased 36 per cent by the end of the third day (Fig. 2, Table V). The esterified cholesterol fraction accounted for about 90 per cent of the over-all decrease. As was the case with the single infusion, free cholesterol increased in whole serum and subnatant reaching a maximum eight hours after the start of the infusion. This pattern remained relatively stable throughout the period. The serum cholesterol and phospholipids returned to preinfusion levels on the sixth day, but the neutral fatty acids remained elevated as is shown in Figures 1 and 2 and Table V.

COMMENTS

The marked differences in serum fatty acids after administering the same amount of fat orally or intravenously deserves some comment. One must remember that the intravenous fat was delivered to the greater circulation at a rate of 94 mEq. per hour and cleared at an average rate of 49.5 mEq. per hour. Although the exact data are unavailable for rate flow of chyle in normal persons after fat meals, incomplete information would suggest that the rate of flow would deliver less than 94 mEq. per hour to the greater circulation. Thus, differences in rate of delivery of fat to circulation would account for the much lower levels of fatty acids seen in the subjects receiving the fat by nasogastric tube. Furthermore, since we have no estimation of digestibility of the fat emulsion or of rate of entry of chyle into circulation we cannot calculate clearance rates for the intragastrically administered fat. There may be reason to believe that fat delivered as chyle may be chemically and physiologically different from artificially prepared emulsion. Studies from this laboratory[9] on composition of serum chylomicron have shown that the infused chylomicrons quickly become associated with protein in amounts which compare favorably with the figures in the literature for natural chylomicrons. Experiments on animals in which artificial chylomicrons were infused have shown sequestration of the fat particles in the reticuloendothelial cells throughout the body which is not true for natural chylomicrons.[10] However, particle size, rate of administration, synthetic emulsifiers and other unknown factors may be responsible for this discrepancy. Evidence is available, however, to show that the syn-

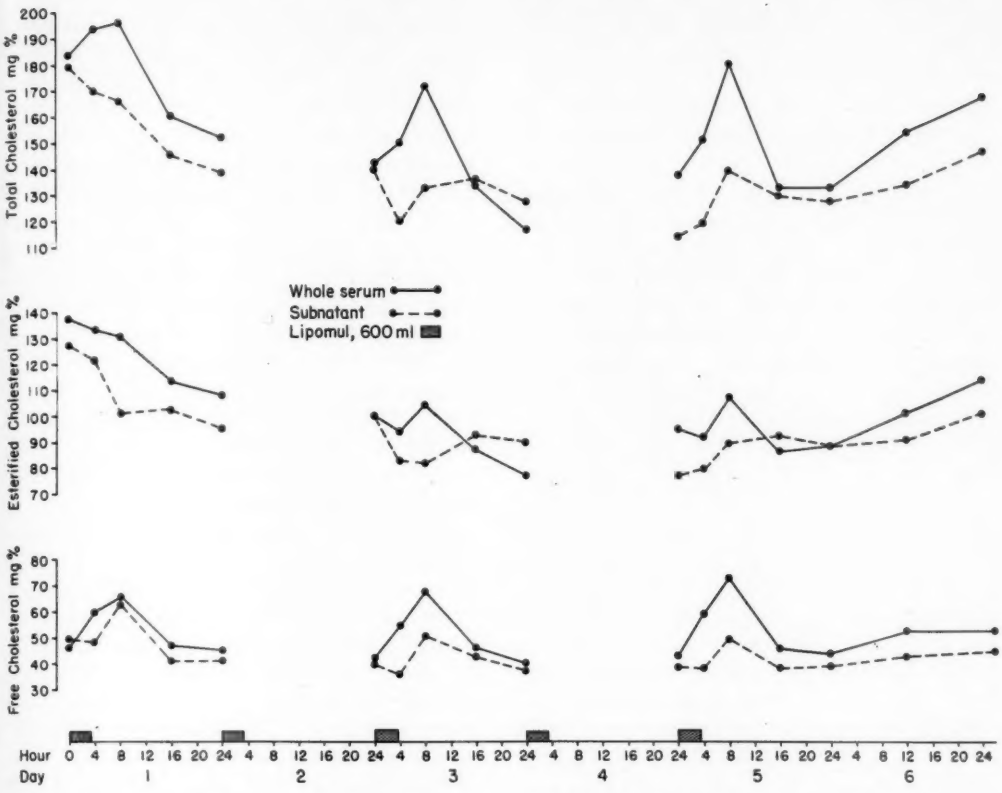

FIG. 2. Average values for total, free and esterified cholesterol in whole serum and subnatant serum of four healthy male subjects on days one, three and five following daily intravenous infusion of fat emulsion.

thetic chylomicron can serve as a potent source of energy[11,12] indicating, therefore, that intravenously administered fat emulsion is efficiently metabolized by the body.

An increase in the neutral fat fraction of the "soluble" lipoprotein fraction of serum has been previously reported by Swank and Wilmot[13] in dogs following fat meals and by Albrink, Man and Peters[4] in humans with lipidoses. The latter group demonstrated soluble neutral fat in lactescent serum in amounts of 10 to 30.4 mEq. per L. despite variations of fatty acids from 16.8 to 478 in whole serum. Jones et al.[14] have also observed increases in low density lipoproteins after feeding fat in human beings and have suggested that these lipoproteins serve as a transport system for absorbed fatty acids. In the present experiment, similar increases in "soluble" or subnatant neutral fat are reported following intravenous fat infusion. The peak in "soluble" neutral fat occurred eight hours after the start of the infusion or four hours after the maximum serum fatty acid level. Since there is a period of lag in the appearance of the "soluble" lipoprotein, it would appear that the intravenously infused chylomicrons left the circulation essentially intact with re-entry of part of the lipid in the soluble state, probably as newly formed lipoprotein. The transformation of chylomicron fat to soluble low density lipoprotein probably occurred primarily in the liver since this organ has been shown to assimilate a large fraction of infused fat[15,16] and also has been shown to affect lipoprotein formation.[17] Further evidence for the intact removal of chylomicrons from serum is suggested from the

results of a clearing factor enzyme study[18] wherein enzyme levels following a single intravenous infusion of fat emulsion in healthy subjects were very low, accounting for the lipolysis of about 5 per cent or less of the removed lipid.

Only slight increases in the subnatant neutral fatty acids were observed when the fat emulsion was given intragastrically, but this does not rule out the possibility that such increases in low density lipoprotein had occurred since it is possible that the first serum sample was obtained after an increase had taken place. In addition, it should be pointed out that information is not available on the turnover rates of neutral fats from serum of human beings, although some data have been reported in dogs suggesting that the turnover rates may be relatively rapid.[19] Certainly, production of non-esterified fatty acids during and after the infusion of the fat cannot alone account for the rise in subnatant neutral fatty acids, since the maximum value of 1.34 mEq. per L. for production of non-esterified fatty acids occurred at the end of the infusion (Table IV) and thereafter fell toward preinfusion levels. In a recent publication, Frederickson, McCollester and Ono[20] demonstrated that retransport of non-esterified fatty acids does not account for the utilization of chylomicrons labeled with C^{14}-palmitate-triglycerides, and suggest that chylomicrons as such are degraded at tissue sites. Havel[21] has reported the effects of high fat meals in normal human subjects and has shown that the increase in serum neutral fat occurred entirely in very low density lipoproteins (chylomicrons included). The less vigorous centrifiguation in our study as compared to that of Havel (30,000 × G for forty-five minutes vs. 105,000 × G for twenty hours) can account for our separation of the "soluble" very low density lipoproten from the insoluble chylomicrons.

Changes in serum phospholipids are somewhat difficult to interpret since a large amount of phosphatide is added to Lipomul as an emulsifier. Furthermore, it has been observed that on centrifugation of Lipomul, a significant amount of phospholipid can be found in the clear subnatant fraction.

Some insight has been gained into the mechanisms whereby serum cholesterol may be reduced in the blood with fat loading. Decreases in serum lipids after intravenous administration of fat emulsion have been demonstrated by Lever and Waddell[22] and by Albrink and Man.[23] The data reported herein corroborate those previously reported. In an attempt to determine whether cholesterol was an integral part of the circulating particulate lipid, samples were selected from this study for chylomicron analyses. The results of these analyses are reported elsewhere.[9] The findings suggest that all of the decrease in serum cholesterol following fat loading can be explained on the basis of the cholesterol content of the chylomicrons leaving the circulation. From the data accumulated in this study, it appears that cholesterol, free and esterified, becomes associated with infused artificial chylomicrons by shifting from high density lipoprotein to low density lipoprotein (chylomicrons included) and leave the blood in this form. Whether this is due to a simple solubility phenomenon or equilibration as has been suggested by some workers[4] or by some other active mechanism is unknown.

The lowering of fasting cholesterol and phospholipids in serum from day to day can be partially accounted for by the high carbohydrate content of the diet fed in this study. The lowering effect of high carbohydrate feeding on serum lipids in human subjects has been shown by Havel.[24] It was interesting to note that serum lipids returned to their preinfusion levels on the day following the administration of the last bottle of fat. This would suggest, at least for a short term study, that certain lipids can be shifted to other body compartments with rapid re-entry into the blood stream when the fat load is terminated.

SUMMARY

1. For the purpose of studying some mechanisms of lipid transport, the effect of fat emulsion on whole serum and subnatant serum lipids after single intragastric and intravenous administration and after daily intravenous infusion was studied in healthy young adults.

2. The level of whole serum neutral fat

after intragastric administration of fat in the same subject did not result in as great an increase as was observed after intravenous administration. Following a single intravenous infusion of fat emulsion, subnatant serum neutral fat and free cholesterol reached a maximum eight hours after the start of the infusion. Increases were observed after intragastric administration of fat emulsion but not to the same degree as after intravenous infusions. During the first day, cholesterol and phospholipids decreased substantially in whole serum and subnatant after both intragastric and intravenous administration of fat emulsion, but returned to preinfusion levels on the second day of the experiment when no fat load was administered.

3. Daily intravenous administration of fat emulsion resulted in essentially similar patterns on the three days studied. Neutral fat and phospholipids peaked at the end of the infusion in the whole serum, and on successive days, the clearing rate of the infused fat emulsion decreased and the fasting level for neutral fat increased. Neutral fat in the subnatant serum reached its highest level four hours postinfusion along with an increase in cholesterol. The esterified cholesterol fraction dropped in serum from the beginning of the experiment and fell to its lowest point by the end of the third day, gradually returning to preinfusion levels by the sixth day.

4. The results of this study strongly suggest that the infused artificial chylomicrons are removed from the circulation essentially intact, reappearing, at least in part, as "soluble" lipoprotein. Serum cholesterol apparently becomes associated with the infused neutral fat thereby accounting for the immediate decrease in serum cholesterol.

5. The experiments described are discussed in the light of current knowledge in lipid transport.

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Nutrition Surveys

Incidence of Caries Among School Children in Bang Chan, Thailand, 1953

With Notes on Other Observations on Dental Conditions Among Children and Adults

GODIVARI S. KAMALANATHAN, M.S.* AND HAZEL M. HAUCK, PH.D.,† WITH THE COOPERATION OF CHUMLONG KITTIVEJA, D.D.S.‡

THIS study of dental health in a rice-producing village in Thailand was conducted as part of a general investigation of food habits and nutritional status, under the auspices of the Cornell Thailand Project.

In accordance with the recommendation of the Joint FAO/WHO Committee on Nutrition,[1] incidence of caries was recorded as a separate item, and was not taken as a criterion of malnutrition. Since the literature on dental caries has been recently reviewed,[2–4] reference to other studies on incidence of caries in human beings will be reserved for later discussion.

SUBJECTS AND METHOD

Collection of Data

All children who were present at the Bang Chan primary school when the dental inspection was carried out in February 1953, were included in this study. One dentist examined the teeth of 226 children, aged seven to fourteen years, using probe and mouth mirror. The record used by the observer included spaces for identifying information[5] and diagrams for deciduous and permanent teeth. Symbols were used to indicate whether or not a given tooth was erupted and the presence of carious lesions or other abnormalities. Although symbols had been alloted to indicate filling or extraction due to caries, these were not needed as no child had either missing or filled teeth. The figures for carious teeth as reported in this study may therefore be compared with figures for DMF teeth found in the literature.

Analysis of Data

The data were grouped according to sex and age at nearest birthday.[5] The number of specific teeth affected were tabulated and totalled for each group. Data on deciduous and permanent teeth were treated separately, but because of the age group studied, information presented is primarily concerned with permanent teeth.

The numbers of children free from caries of deciduous teeth, of permanent teeth and totally

From the New York State College of Home Economics and Southeast Asia Program, Cornell University, Ithaca, New York.

* Lecturer in Home Science, Sri Avinashilingam Home Science College (Madras University), Coimbatore, India. † Professor of Food and Nutrition, New York State College of Home Economics, Cornell University. ‡ Present address: c/o Faculty of Dentistry, The University of Queensland, St. Lucia, Brisbane, Australia.

The field study was carried out under the direction of Dr. Hauck, who received an award for advanced research from the United States Educational (Fulbright) Foundation in Thailand, 1952–53.

TABLE I
Children Free from Caries of Deciduous Teeth and Caries Incidence in Deciduous Teeth

Age (yr.)	No. Examined*	No. Free from Caries	Total No. of Deciduous Teeth	No. of Carious Teeth	
				Total	Mean per Child
Boys					
7	5	1	75	27	5.4
8	18	8	268	29	1.6
9	9	5	112	12	1.4
10	21	10	224	28	1.3
11	21	16	173	19	0.9
12	14	8	97	18	1.2
13	5	4	9	1	0.2
Total	93	52	958	134	1.4
Girls					
7	8	2	138	22	2.8
8	16	7	231	42	2.6
9	8	2	96	18	2.2
10	11	5	82	17	1.5
11	20	12	110	13	0.7
12	12	7	60	17	1.4
13	5	5	15	0	0.0
Total	80	40	732	129	1.5

* This number does not include the children whose deciduous teeth were all exfoliated.

free from caries were noted. With respect to incidence of caries, the following were calculated:

(1) caries index, i.e., the number of carious teeth per child (comparable to "DMF index");

(2) percentage of deciduous and of permanent teeth found to be carious;

(3) average number of carious teeth among children with caries experience (comparable to "DMF per DMF individual");

(4) extent of caries, i.e., percentage of children with each number of carious teeth, as 0, 1, 2, 3, and so on; and

(5) relative incidence and distribution of caries, or the contribution toward caries by each pair of corresponding teeth in each jaw, as percentage of the total number of carious teeth. In calculating this item, the data for all ages were combined.

RESULTS

None of the children had received professional dental care, and little attention was paid to mouth hygiene. Brushing of teeth was not usual, but the practice of rinsing the mouth with water after meals was relatively common.

Incidence of Caries among School Children

When observations on deciduous and permanent teeth were combined, 45 per cent of the boys and 38 per cent of the girls were found to be totally free from caries. The average number of caries of both types of teeth combined was 1.8 and 2.0 per boy and girl, respectively.

Deciduous Teeth: Of the children who still had deciduous teeth, half of the girls and more than half of the boys had no caries in such teeth. The average number of caries of deciduous teeth among children who had experienced caries was found to be 3.2 for both boys and girls. Of forty-one boys and forty girls who had caries of deciduous teeth, twenty-nine boys and thirty girls had less than four carious teeth each. Only three boys and two girls had more than eight carious deciduous teeth each. First and second molars contributed 76 and 83 per cent of the total caries in boys and girls, respectively. Observations on caries in deciduous teeth are summarized by age and sex in Table I.

Permanent Teeth: For all ages, 60 per cent of the girls and 74 per cent of the boys were free from caries of permanent teeth. For boys and girls 3 and 5 per cent, respectively, of the permanent teeth were found to be carious and the number of carious teeth among those who had caries of the permanent teeth was 1.9 and 2.3, respectively.

The number of children examined, the number free from caries, and the number of carious permanent teeth noted are shown for each age group in Table II. Among the thirty boys who had carious permanent teeth none had more than four, while among forty-three girls with experience of caries only five had above four carious teeth each. In boys the first

TABLE II

Children Free from Caries of Permanent Teeth and Caries Incidence in Permanent Teeth

Age (yr.)	No. Examined	No. Free from Caries	Total No. of Permanent Teeth	No. of Carious Teeth	
				Total	Mean per Child
Boys					
7	5	4	32	1	0.20
8	18	15	141	3	0.22
9	9	8	96	1	0.11
10	21	17	290	9	0.38
11	25	15	444	18	0.72
12	20	15	423	15	0.75
13	17	11	447	9	0.54
14	1	1	28	0	0.00
Total	116	86	1901	56	0.50
Girls					
7	8	6	44	3	0.37
8	16	15	144	1	0.06
9	8	6	86	2	0.25
10	12	8	205	9	0.75
11	24	12	474	27	1.10
12	19	8	425	26	1.30
13	19	9	501	24	1.27
14	4	3	112	6	1.50
Total	110	67	1991	98	0.90

and second molars of the lower jaw contributed 50 and 32 per cent, respectively, toward total incidence of caries. The corresponding teeth in the upper jaw contributed 7 and 4 per cent, respectively. Among girls, 49 and 22 per cent of teeth attacked by caries were the lower first and second molars and 6 and 9 per cent, upper first and second molars.

COMMENTS

The low incidence of caries among these Thai children appears even more striking in contrast to caries incidence among children in the United States. As an illustration, the number of DMF teeth per 100 erupted permanent teeth is shown for two age groups in Newburgh and Kingston, New York,[6] as compared to Bang Chan (Table III). In the Newburgh-Kingston caries-fluorine study, both clinical and roentgenographic examinations were used to detect caries, but for comparison with our observations in Bang Chan, only the information obtained from clinical study is shown. The lower incidence of caries among children in Newburgh than in Kingston, New York, is attributed to fluoridation of the drinking water which had been in effect in Newburgh for ten years. The much lower incidence of caries in Bang Chan, where rain water or surface water is used for drinking,[7,8] than in Newburgh, suggests that some unidentified factors operating in Bang Chan are even more effective in control of caries than fluoridation. In an isolated village in Greece, where school children were found to have extremely low incidence of caries, the stored rain water which was used for drinking was found to contain only 0.03 p.p.m. of fluorine.[9] Hurtarte and Scrimshaw[10] reported relatively low fluorine content, 0.4, 0.5 and 0.7 p.p.m., in the water supplies of three Guatemalan villages where incidence of caries was low among school children. Clearly, a fluorine content of 1 p.p.m. or more in the drinking water is not essential to relatively low incidence of caries in children. In Bang Chan, although samples of drinking water were not analyzed, one would expect the fluoride content of the rain water and surface water to be low, and prevention of caries from this source seems unlikely.

The abundant sunshine, to which all children in Bang Chan were exposed after early infancy, may have been a factor in resistance to caries. In central China, however, where there is little sunshine during the long winter

TABLE III

DMF Teeth per 100 Erupted Permanent Teeth (Clinical Examination)

Age (yr.)*	Newburgh	Kingston	Bang Chan
10–12	13.9	26.3	4.6
13–14	19.9	34.4	3.6

* In Newburgh and Kingston this represents age at last birthday, whereas in Bang Chan, the age at nearest birthday was used.

months, Afonsky[11] reported low incidence of caries. Although a number of studies where low incidence of caries was found have been conducted in areas of relatively abundant sunshine, high incidence of caries has been noted in the Virgin Islands[12] and the Panama Canal Zone.[13] In both instances, the investigators mentioned low fluorine content of rain or lake water used for drinking as probable major causes of susceptibility to caries. As has been noted, however, low fluorine content of drinking water is not incompatible with low incidence of caries.

Considering diet, the nutritive value, texture, carbohydrate content and particularly the presence or absence of sugar in a form likely to adhere to the teeth have been considered as affecting the incidence of caries. The controversial nature of the part played by diet in the production of teeth which are unduly susceptible to caries was noted by the Joint FAO/WHO Committee on Nutrition,[1] although as they stated, in general, the importance of nutrition, especially in the formative period, is widely accepted. Nevertheless, resistance to caries occurs in some populations whose diets appear to be grossly inadequate by current standards.[10] The diet in Bang Chan appeared to be very low in certain nutrients, notably calcium, riboflavin, thiamine, vitamin A value and probably in linoleic acid,[8,14] and over half of the school children had possible signs of malnutrition. Dental development, however, was similar to that of children in other areas,[5] and incidence of caries was low despite the poor diet.

Some investigators have suggested an association between the abrasive action of hard foods,[9] grit,[15] and chewing hard "betel nuts,"[16] and the low caries incidence noted in an area. Smoothing of pits and fissures were thought to decrease formation of caries. In Bang Chan, however, the diet was markedly soft and pappy, and no abrasion was noted on the teeth of school children. Afonsky[11] has also commented on the fact that the soft Oriental diet, with its high rice content, did not predispose to caries.

Several reports have appeared of higher incidence of caries among children whose diets were subject to Western influence, in areas where children were otherwise relatively caries-free. Thus, Shourie[17] found the highest incidence of caries in permanent teeth among 6,866 children examined, in an Anglo-Indian orphanage where the diet was in many respects superior to that of Indian children, but included bread from refined wheat flour and fairly large amounts of sugar. Kridakara et al.[18] noted that children in an American missionary school in Lampang had a higher rate of caries attack than others, and that this was largely responsible for the elevated value reported for young children in Lampang. These authors also suggested that the high rate of caries attack in children living in Bangkok might be due in part to increased consumption of sugar and wheat flour. They noted that in Bangkok "the basic Thai diet of rice, fruit, and fish has been replaced in part or supplemented by sugar and starch-containing foods and confections." Losee[19] noted a large difference in incidence of caries among students on one island in Samoa, and commented that the DMF rate roughly paralleled the total merchandise sold in village stores. Although Losee did not focus special attention on refined carbohydrates, the list of foods sold in stores in 1950 included an average of 41 gm. patent flour, 32 gm. sugar, 3 gm. candies and 0.6 gm. sweet drinks per capita per day. Consumption of such foods might be relatively high in the area most accessible to the store. Losee pointed out the value of studying dental conditions in a situation such as this, where some persons live much as they did before Western influence reached the island, while the habits of others have been markedly changed by Western contacts.

Afonsky observed no case of acute or rampant caries among 3,349 students observed in central China. He stated that cases in which permanent teeth were so affected were unknown in that part of China. They were seen, however, among children with deciduous teeth, and all such children had a history of constant use of candy "pacifiers." Changes in consumption of sugar have been associated with a decreased rate of dental caries among Norwegian school children during World War

II and a subsequent increase in postwar years.[20] A cause and effect relationship, although it appears likely, has not been clearly proved.

Two recent studies on the relation of intake of sugar to caries by Gustafsson et al.[21] and King et al.[22] have received much attention. In the former, an extensive study on adults in an institution, the detrimental effect of sugar in a form which would adhere to the teeth was demonstrated. Even larger amounts of sugar in bread eaten at meals did not increase the rate of caries above that on the usual diet. From the latter study, King et al. concluded that "while this investigation does not prove that the sugar content of the diet, *however distributed and however eaten*, never affects the teeth, it certainly points to the practical conclusion that, provided a child is fed on a diet as good as that of the average children's residential institution in England, a substantial increase in the amount of sugar in the diet will not affect the child's liability to dental caries within a period of at least 2 years." We have no way of knowing to what extent the sugar present in the basal diets of these children contributed to the substantial incidence of caries among them. The subjects in these groups ranged from four to fourteen years of age and may therefore be compared to the subjects of the present study. In Liverpool the average consumption of sugar on the basal diet was 15 oz. per week; in Sheffield, $19^1/_2$ oz. in winter and $23^1/_2$ oz. in summer, i.e., the control groups received on the average from about 61 to 95 gm. of sugar per day.

Each child in the "sugar groups" received an additional 22 oz. of refined white sugar per week, so that the total, an average of 150 to 184 gm. per day, approximated (or sometimes exceeded) prewar consumption. This is even higher than the estimated mean per capita consumption of sugar at the retail level in the United States, which has ranged between 123 and 137 gm. per day for the years 1949 to 1957.[23] In the study by King et al., when data for the children in Liverpool and Sheffield studies were combined, 31 per cent of first permanent molars were carious in fifty-six control children seven to ten years old, as compared to 5.8 per cent carious first permanent molars in the same age group in Bang Chan.

After one and a half years of study, these same control groups of children in Liverpool and Sheffield had 52.1 per cent of first permanent molars carious as compared to 13.4 per cent in children of the nine to twelve year age groups in Bang Chan, where consumption of sugar was very low. For eleven families whose consumption of food was observed on four survey days each, the mean per capita daily intake of sugar was about 8 gm. The highest per capita intake, for one family, was 28 gm. per day. The range for the remaining ten families was from about 1 to 14 gm. per capita per day. Palm sugar was generally used as flavoring in curries, not as sticky sweets.

So far as we know, no conclusive evidence exists that low intake of sugar is an important factor in low incidence of caries in human beings, yet one cannot overlook the fact that low intake of sugar (but not necessarily low intake of total carbohydrate or of refined carbohydrate-rich foods such as polished rice) has been common to groups of people in various areas where very low incidence of caries has been reported[9,10,11,15,17,18] and high intake of sugar has been common where reported incidence of caries is high.[12,13] Further studies of groups such as this one in Bang Chan, in comparison to a group such as the Thai children, only twenty miles away in Bangkok, where Kridakara et al.[18] found a much higher incidence of caries, should help to solve the riddle of dental caries. In these two situations, differences in race, climate and sunshine are minimized. If differences in kind and amount of various foods eaten are critical, chances of identifying the foods or nutrients of importance in the carries problem would be greater where the basic food supplies of the two areas to be compared are similar.

Detailed study of diets in places where incidence of caries is even lower, as in the isolated village in Greece studied by Philippas[9] would also be of value. Philippas examined seventy-nine of eighty-two children four to fifteen years old, in this village. Of these, sixty-eight,

or 86 per cent, were completely caries-free, and the average number of caries in both deciduous and permanent teeth was only 0.25 per child. In Bang Chan, if boys and girls are considered together, 42 per cent were totally caries-free, and the average number of carious teeth for deciduous and permanent teeth, combined, was 1.9 per child. More detailed study of diet in relation to dentition should be undertaken in areas such as these while the opportunity remains.

Other Observations on Teeth and Gums of School Children

Stain and Calculus: Teeth of almost all children were stained, but in most staining was slight. About 40 per cent of the children had calculus, which was slight in the majority of cases.

Developmental and Other Abnormalities: Other abnormalities of the teeth were noted in seventy-five children. About three-quarters of these were accounted for by linguoversion, torsiversion, labioversion and diastema, with other irregularities such as crossbite, deep bite, supernumerary teeth and peg-shaped teeth occurring less frequently. Only one fractured tooth was seen and in none was abrasion noted.

Condition of Gums: About 30 per cent of the children had redness of gums, but the gums were swollen in only two instances. Retraction or loss of interdental papillae was not seen. The dietary records that had been obtained from eighty-three of the children in the third and fourth grades were checked for consumption of fruits and vegetables. From these qualitative records children whose gums were red did not appear to eat foods rich in ascorbic acid less often than those whose gums were of normal color, but this does not mean that no relation exists between low intake of ascorbic acid and redness of gums in these children. Intakes of ascorbic acid in families that took part in the quantitative dietary study[14] were adequate to prevent scurvy, but not liberal. The estimated average per capita intake of ascorbic acid was about 25 mg. per day, with a range of 3 to 95 mg. on forty-four survey days.

NOTE ON DENTAL STATUS OF ADULTS IN BANG CHAN

Relative freedom from caries in children does not necessarily mean that the teeth of adults in the same population will be in good condition. Thus, Hurtarte and Scrimshaw[10] commented, concerning the situation in three Guatemalan villages where the school children were relatively free from caries, that "... it is apparent to anyone working in the villages that adult dental status is very poor. This may be due to the neglected accumulated effects of a relatively low rate of caries, differences in dietary and other habits in the adults, or poor dental hygiene in any combination."

In Bang Chan the one conspicuous difference in food habits of school children and adults was that snacks, including fruit, were eaten primarily by children. Dental hygiene was not of much concern to either children or adults. None of the school children had been to a dentist, and dental work done on the teeth of adults appeared to be largely for decorative purposes. The practice of betel chewing, which was habitual with many adults in Bang Chan, might have an effect on the health of teeth and oral tissues. Therefore, the information obtained on the age at which loss of teeth occurred, prevalence of caries, the practice of crowning teeth and the habit of betel chewing among adults in Bang Chan, is presented.

Subjects and Method

Members of thirty-one households, chosen at random, were given a medical inspection by a physician, which included examination of the mouth and teeth. Ninety-eight persons over fifteen years of age, forty-five males and fifty-three females, were examined. This inspection was less detailed than that carried out by the dentist on the school children. The number of teeth present in adults, the condition of teeth and whether or not the subject chewed betel were recorded. If teeth had been crowned, the physician recorded whether crowning was done on a sound tooth or in place of a missing one.

Number of Teeth Present in Adults

Among the adults examined in Bang Chan

TABLE IV
Mean Number of Teeth Present in Forty-five Men and Fifty-three Women Over Fifteen Years of Age

Age Group (yr.)	No. Examined	Mean No. of Teeth per Person
Males		
15–24	16	30.9
25–34	6	30.1
35–44	13	30.6
45–54	5	28.2
55–64	4(1)*	12.2(16.3)†
Over 65	1	25
Females		
15–24	13	30.3
25–34	10	31.6
35–44	10	31.0
45–54	9	23.6
55–64	5(3)*	6.4(16.0)†
Over 65	6(2)*	2.7(4.0)†

* Number of edentulous persons in the group is shown in parenthesis.

† Mean number of teeth per person, when edentulous persons were not included.

most retained almost a full complement of permanent teeth up to forty-four years of age (Table IV). Of sixteen adults between the ages of fifty-five and eighty-two, six were edentulous, and most of the others had lost many teeth.

The Practice of Crowning Teeth

Of the ninety-eight adults examined, eight had crowns in place of sixteen missing teeth, whereas eighteen persons had a total of thirty-eight sound teeth crowned. The fact that in both sexes only front teeth were crowned while carious teeth in other areas were not filled or crowned strengthens the impression that crowning was practiced primarily for decorative purposes.

Incidence of Caries in Adults, and Betel Chewing in Relation to Dental Health

Among adults in Bang Chan caries did not appear to be prevalent. In the age group twenty-five to forty-four years, carious teeth or crowns which replaced missing teeth were noted in only five of twenty men and five of eighteen women. Of the ninety-eight persons over fifteen years of age who were examined, forty-eight were betel chewers, but none of the habitual betel chewers were under twenty-five years of age. As the adults under forty-five years of age retained nearly all of their teeth, the age group twenty-five to forty-four years was selected for comparison of teeth of betel chewers with non-chewers. Of twenty men in this age group, no carious or crowned missing teeth were noted in the six betel chewers, whereas five of the fourteen non-chewers had such defective teeth. Among eighteen women, defective teeth were noted in only three of thirteen betel chewers, and in two of five non-chewers. When the men and women were considered together, seven of the nineteen non-chewers had a total of forty-six defective teeth, whereas among the nineteen betel chewers, only five defective teeth were noted, in three persons. Because of the small number of adults observed, and the likelihood that some caries in betel chewers may have been overlooked because the teeth were not cleaned before the inspection, caution must be used in interpreting these observations. In Guam, where some children begin to chew betel at about eleven years of age, Gerry et al.[24] found fewer dental caries among the betel chewers than non-chewers. Shourie[16] found that Indian children in a region where betel chewing may be started as early as three or four years of age had a lower incidence of dental caries than children in regions where betel chewing was less prevalent.

The protruding incisors which Balendra[25] described as the result of loosening teeth and malocclusion in betel chewers, were seen frequently among the middle-aged and older betel chewers in Bang Chan. Such protruding incisors were not observed among the children, or among non-betel chewing adults.

Whether or not betel chewing should be discouraged is controversial. Even where spitting the juice is usual, as in Bang Chan, some of it is probably swallowed, and the calcium value[26] of lime used on piper betel leaves might be of importance to adult women in an area where calcium intake is low. The

available evidence suggests that although the practice of betel chewing may inhibit formation of caries, it is detrimental to gums and supporting tissue.

SUMMARY

The teeth of 226 children seven to fourteen years of age attending the primary school in Bang Chan, Thailand, were examined by one dentist. Among these children incidence of dental caries was low. In the entire group 42 per cent were totally free from caries. The average number of carious teeth, both deciduous and permanent, per child was 1.9. When deciduous teeth alone were considered, 56 per cent of the boys and 50 per cent of the girls were free from caries. In the whole group, 74 per cent of boys and 60 per cent of girls were free from caries of permanent teeth.

The reason for this low incidence of caries is not known, but in common with people in other areas where very low incidence of caries has been reported, the intake of sugar of residents of Bang Chan was very low.

The teeth of ninety-eight persons over fifteen years of age were examined by a physician in the course of medical inspection of a random sample of families in the community. Of these, sixty-eight persons up to forty-four years of age retained almost a full complement of teeth. Most older persons examined had lost a number of teeth and some were edentulous.

Some adults had missing teeth crowned. The services of a dentist appeared to have been sought more for cosmetic reasons, however, than to restore missing teeth, as a greater number of persons had sound teeth rather than missing teeth crowned.

The possible relation of the practice of betel chewing which was habitual in about half of the adults is discussed. Protruding incisors were seen frequently among middle-aged and older persons who chewed betel.

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A Dental Survey of a Small Group of Thai Children

KALYANI AMATAYAKUL, D.D.S.,* M. R. NIBHASARA LADAVALYA, D.D.S.† AND ROBERT HARRIS, M.D.S.‡

A SURVEY was undertaken by Kridakara et al.[1] in 1951 which provided data on the prevalence of dental caries in 2,300 Thai children and on their occlusal and gingival states. About half of these children were from the capital city of Bangkok and the remainder were from the provincial city of Chieng Mai and from Lampang in the northern hill country. The two latter centres are close together and about 700 kilometres north of Bangkok; although access is by no means difficult, they are not affected quite so much by the impact of western civilisation as is Bangkok. Nevertheless, there is a not inconsiderable increase in the extent of this impact at the present time (1958).

Since one of us had participated in a long term survey[2] of a selected group of boys and girls living in Bowral, New South Wales, who had a low prevalence of dental caries associated with a dietary regime marked by the absence of refined carbohydrates and meat (described in detail by Gilham and Lennon[3] and which is a lactovegetarian diet), the opportunity was taken to make a survey of a similar age group of school children in Chieng Mai province. The examination was identical in every respect with that carried out in Australia, and the same examiner made the observations in both surveys. The examination included (1) observations on the extent of dental caries, (2) bacteriologic examinations of the saliva (3) a qualitative analysis of the dietary program, and (4) prevalence of gingivitis and calculus.

This report will cover the first three sections.

* Associate Professor of Bacteriology; † Associate in Operative Dentistry, School of Dentistry, Bangkok, Thailand. ‡ Head, Department of Preventive Dentistry, United Dental Hospital, Sydney, Australia.

The work reported here represents part of a survey carried out in 1958 when one of the authors (R.H.) was serving as a WHO Consultant in Dental Health to the Government of Thailand.

METHODS AND MATERIALS

Four groups of children ranging in age from nine to sixteen years were examined. These groups consisted of school children attending Thai boys and girls schools in Chieng Mai and a mixed school in Sarapi Village, distant about 15 kilometres from Chieng Mai. Chieng Mai is the capital of the province of the same name, is the terminal of the northern railway system, the centre of various cottage industries and a centre for the marketing and milling of grain, timber and agricultural products. Sarapi is a village centre for agricultural workers.

The clinical examination was made in good light with a standard mirror and sharpened probes, identical in size and shape with those used in the Bowral surveys. A bite-wing radiographic survey of every child was made, with, wherever necessary, x-ray films of the anterior teeth. The clinical examinations were made by one examiner (R.H.) and the results of the examinations were subsequently compared with the radiographic examination.

Saliva was collected at a set time (10:30 A.M.) by the standard method of vigorously chewing paraffin wax and expectorating the saliva into a numbered sterile screw top bottle for a five-minute period. The bottles were then sealed with adhesive tape, placed in special fibreglass containers transported by air freight to Bangkok where they were collected and stored overnight in a refrigerator. Twenty specimens were sent in each batch as this was

the maximum number which could be properly processed in Bangkok. In order to maintain the saliva at an even temperature during the flight, the bottles were cooled in a refrigerator and then placed in the containers below a layer of crushed ice. Just prior to despatch the ice was removed and the space packed with cotton wool. In this way all the saliva arrived without damage and the bacteriologic procedures could be commenced as soon as the specimen had been allowed to reach room temperature.

The dietary record was obtained by interviewing each child and explaining the record sheet and method of recording the data. Each child was asked to record daily, by a stroke in the space provided, the number of servings of each item of food eaten. In order to test the comprehension of the child and to seek his cooperation, a record of the previous day's food was made, with the child's help, and checked by careful questioning so that each child was familiar with the information required and the method of recording it. The chart was designed to give the information in five food groups, and to facilitate evaluation an English translation of the name of each food was listed with the Thai language equivalent. Figure 1 shows the English translation.*

Name_____ Birthday_____ Sex____ No. ____

Please mark in the appropriate column with a ✓ the number of servings of foods each day.

	Food Groups	Days 1	2	3	4	5	6	7	Total
Protein	Pork, chicken, beef								
Fat	Egg								
Carbohydrate	Lard, coconut								
	Rice								
	"Glutinous" rice								
	White noodle								
	Yellow noodle								
Fruits and Vegetables	Banana								
	Orange, tomato								
	Pineapple								
	Papaya								
	Mangkut								
	Jack fruit								
	Pumpkin								
	Leaf vegetable								
	Bean, bean sprout								
Confectionery	Ice cream								
	Coconut milk								
	Lollies								
	Toffee								
	Coca-Cola								
	Condensed milk								
	Food in syrup								
	Total								

FIG. 1. Form of diet record sheet used in survey of Chieng Mai and Sarapi Village school children.

RESULTS

Table I shows the age groups of the children and the prevalence of dental caries. Of the eighty children, forty-two (52.5 per cent) were found to be caries-free (forty-seven [58.25 per cent] had no caries in the permanent dentition). Thirty-seven children had mixed dentitions and of these, twenty were caries-free. Of the forty-three children with permanent dentitions, twenty-two were caries-free.

The average prevalence of dental caries per child (for all the permanent teeth) expressed as DMF teeth† is:

(1) All children, 1.12 DMF teeth;
(2) Chieng Mai children, 1.97 DMF teeth;
(3) Sarapi Village children, 0.27 DMF teeth.

Since there is always a difficulty of expressing accurately the prevalence of dental caries in the deciduous dentition, we have used a figure which shows the percentage of carious deciduous teeth, the df‡ teeth per 100 deciduous teeth as follows:

(1) All children, 13.8 per cent;
(2) Chieng Mai children, 19.4 per cent;
(3) Sarapi Village children, 9.1 per cent.

The assessment of the experience of dental caries, using bite-wing films as a further aid, demonstrated six carious permanent teeth and one carious deciduous tooth, missed by clinical examination, but also eliminated eight deciduous teeth marked as having carious lesions on proximal surfaces.

* The dentist from Chieng Mai Hospital and class teachers cooperated in additional explanation and supervision of the daily food record.

† D = decayed; M = missing (extracted because of disease); F = filled in the permanent teeth.

‡ d = decayed; f = filled in the deciduous teeth.

TABLE I

Prevalence of Dental Caries of Eighty Thai Children Aged Nine to Sixteen Years

Age Group (yr.)	Average Age (yr. and mo.)*	No. of Children	No. of Mixed Dentitions	No. of Permanent Dentitions	Carious Teeth		Average Carious Teeth		Caries-Free Mouths	
					df†	DMF	df*	DMF*	Mixed Dentitions	Permanent Dentitions
Chieng Mai										
Girls:										
9–11	10.6(6.4)	10	8	2	20(60)	15	2.0(2.4)	1.5(1.75)	2	0
12–14	13.10(5.5)	6	..	6	...	25	...	4.16(5.0)	..	0
15–16	15.3(5.2)	4	..	4	...	5	...	1.25(1.7)	..	2
Boys:										
9–11	10.4(7.9)	9	4	5	1(30)	15	0.1(0.1)	1.66(2.5)	2	3
12–14	12.3(5.5)	11	5	6	0(18)	19	...	1.81(2.34)	3	1
Boys and Girls										
9–16	13.2(16.0)	40	17	23	21(108)	79	0.52(2.14)	1.97(2.9)	7	6
Sarapi Village										
Girls:										
9–11	10.4(8.7)	15	9	6	5(72)	9	0.33(0.97)	0.6(0.95)	5	4
12–14	12 11(5.4)	5	..	5	...	1	...	0.2(0.38)	..	4
Boys:										
9–11	10.1(10.0)	7	6	1	7(44)	0	1.0(1.2)	0(...)	3	1
12–14	12.11(10.0)	13	5	8	0(15)	1	0	0.07(0.33)	5	7
Boys and Girls										
9–14	12.1(15.4)	40	20	20	12(131)	11	0.30(1.79)	0.27(0.48)	13	16

* Standard deviations are given in parentheses.

† Figures in parentheses in this column are total number of deciduous teeth.

Table II shows the results of the dietary surveys, the range of lactobacillus acidophilus estimations and the Snyder colorimetric tests, and the relation between these diagnostic aids and the presence or absence of dental caries.

By relating the bacteriologic examinations of the saliva with the presence or absence of dental caries, fourfold tables were constructed which showed that only in the Chieng Mai children was there some relationship between the presence of lactobacilli and dental caries, and it is slightly significant ($\chi^2 = 2.887$, $P < 0.10$). The correlation between the Snyder test and dental caries is poor in all groups, P being greater than 0.70 in each case. The limitations in using the single lactobacillus acidophilus counts and Snyder tests as diagnostic aids in assessing dental caries activity is recognised and is discussed later.

Table II also shows the general pattern of the eating habits of these children; fruit and vegetables were consumed somewhat more extensively than other food groups, the girls in both groups had more servings than the boys and confectionery was consumed much less by the village children than by those from the city.

TABLE II

Diet Pattern and Prevalence of Dental Caries Compared with Saliva Examinations of Seventy-Four* Thai Children

Age Group (yr.)	No.	Average Servings of Food During Five-Day Period						Prevalence of Caries		Lactobacillus Count			Snyder Test		
		Protein	Fat	Fruits and Vegetables	Carbohydrates	Confectionery	Total	df Teeth	DMF Teeth	0 to 999†	1,000 to 9,999	> 10,000	−	+	++
Chieng Mai															
Girls:															
9–11	9	15.2	3.7	13.2	13.3	5.2	50.6	1.8	1.5	6	..	2	4	..	4
12–14	5	11.5	2.5	12.3	11.6	9.6	47.5	...	4.8	3	2	1	1	1	4
15–16	4	14.0	4.5	18.7	12.7	8.2	58.1	...	1.25	4	..	..	2	2	..
Total	18	13.7	3 5	14.0	12.6	7 3	51 3	1.0	2.25	13(11)	2	3	7	3	8
Boys:															
9–11	9	13.8	3.4	13.7	11.4	5.3	47.2	0.1	1.66	5	3	1	3	1	5
12–14	10	12.1	2.4	12.7	12.4	5.6	45.2	0	1.81	6	3	1	2	2	6
Total	19	12.4	2.8	13.2	12.0	5.5	48.9	0.05	1.7	11(10)	6	2	5	3	11
Sarapi Village															
Girls:															
9–11	14	15.5	2.8	20.1	15.0	3.1	56.5	0.33	0.6	13.	1	..	2	3	11
12–14	5	14.0	2.4	26.0	13.8	4.5	61.6	...	0.2	3	1	1	2	..	1
Total	19	15.1	2.7	21.6	14.6	3.7	57.8	0.26	0.52	16(11)	2	1	4	3	12
Boys:															
9–11	6	12.6	1.0	12.5	12.5	3.0	43.6	1.0	0.0	3	2	..	3	1	2
12–14	12	12.3	1.4	16.2	16.1	1.1	47.1	0	0.07	8	2	3	6	1	5
Total	18	12.4	1.4	15.5	15.0	2.4	46.8	0.38	0.05	11(9)	4	3	9	2	7

Correlation

Group	Lactobacillus				Snyder Test			
	Present		Absent		Negative		Positive	
	Caries	Caries-Free	Caries	Caries-Free	Caries	Caries-Free	Caries	Caries-Free
Chieng Mai‡	15	3	11	10	6	3	20	10
Sarapi Village§	5	15	6	14	3	10	8	19
All children‖	20	18	17	24	9	13	28	29

* The smaller number arises because observations have been recorded on those children who returned satisfactorily completed records.

† Figures in parentheses are numbers of negative counts.

‡ Lactobacillus: $\chi^2 = 2.887$; $P < 0.10$. Snyder: $\chi^2 = 0.131$; $P > 0.70$.

§ Lactobacillus: $\chi^2 = 0.268$; $P > 0.50$. Snyder: $\chi^2 = 0.007$; $P > 0.95$.

‖ Lactobacillus: $\chi^2 = 1.209$; $P > 0.30$. Snyder: $\chi^2 = 0.005$; $P > 0.95$.

TABLE III

Relation Between Food Groups and Experience of Dental Caries in Seventy-Four Thai Children

Group	No.	Average Servings of Food Groups for Five Days			Average Prevalence of Dental Caries	
		Carbohydrate*	Confectionery	Fruits and Vegetables	df Teeth	DMF Teeth
Chieng Mai						
Girls	18	12.6(4.9)	7.3	14.0	1.0	2.2
Boys	19	12.0(6.2)	5.5	13.2	0.05	1.9
Sarapi Village						
Girls	19	14.6(11.8)	3.7	21.6	0.26	0.52
Boys	18	15.0(12.3)	2.4	15.6	0.38	0.05

* Figures in parentheses show servings of glutinous rice.

Table III has been prepared to show this pattern and the prevalence of dental caries. There is a further difference in the composition of the carbohydrate foods (a distinction is here made separating confectionery and carbohydrate foods). The consumption of glutinous rice* is much higher in the children from the rural area. It is a custom which we observed commonly in the rural area among children of all ages.

A comparative picture of the experience of dental caries of these children may be seen in Table IV in which the data on the experience of dental caries of the Thai children, those from the Hopewood House[2] and those from the State School (N.S.W.)[4] are given. The age group nine to thirteen years is presented because this age range is common to all three studies. It will be seen that there is a low experience of dental caries in the Thai children, and that the children from the Hopewood House have a similar low experience.

* Glutinous rice as eaten in this area is unrefined, frequently pounded in the home. It is prepared for eating by soaking the raw grain for about twelve to seventeen hours in water. The water is poured off and the rice is steamed until cooked. As its name implies, it has a higher glutin content than other forms and when prepared has a greyish colour; the grains stick together. When chewed, the bolus of food does not readily adhere to the tooth surfaces and in the many samples examined seemed to have a texture more like coconut.

COMMENTS

The general pattern of the two groups of Thai children is clear. The prevalence of dental caries of the children in Chieng Mai based on the average DMF teeth is seven times that of the children from Sarapi Village: the percentage of carious deciduous teeth of the children in Chieng Mai is twice that of the children from Sarapi Village. In the case of the permanent teeth in the girls, it should be noted for the nine to eleven year age groups the differences are not so marked and in the twelve to fourteen year age groups where there appears to be a marked difference the two groups have a marked difference in the standard deviations. The two groups combined have about the same prevalence of caries as those children of similar age having a diet free of refined carbohydrates living in a home (Hopewood House Bowral, N.S.W.) and a very much lower prevalence of dental caries than a group of comparable age from schools in metropolitan and rural areas in New South Wales. Table IV shows this difference to be very marked in the thirteen year old children.

Only a limited interpretation should be given to the lactobacillus counts, since single deter-

TABLE IV

Comparison of the Average Number of DMF Teeth of Sixty-Nine Thai Children with That of Similar Age Groups of Children at Hopewood House, Bowral[2] and Attending State Schools in New South Wales[4] (Average DMF Teeth for Each Age Group)

Group	Age (yr.)				
	9	10	11	12	13
Thai children	0.1(0.31)	1.64(2.23)	0.83(1.26)	0.74(1.62)	0.37(0.51)
Hopewood children	0.41(0.84)	0.38(0.82)	0.61(1.28)	1.08(1.90)	1.06(1.78)
State School children (N.S.W.)	4.44(2.32)	5.28(3.08)	6.98(4.10)	9.32(5.33)	10.70(5.15)

NOTE: Standard deviations are in parentheses.

minations may vary not only in the one individual[5] but also in aliquot parts from the same sample[6] in regard to any significance in relation to their magnitude and the pattern of dental caries existing at the time of the observations. However, Snyder colorimetric tests showed correlation with lactobacillus counts to the extent that when the latter was above 1,000 the Snydet test was 2 plus. The corollary to this did not hold, especially with the Sarapi Village girls in which group twelve had Snyder 2 plus but only three of these had lactobacillus counts above 1,000. Goldsworthy[7] has drawn attention to this problem and says "the question of duration of the 'infected' state of the mouth by *Lactobacillus* spp. is liable to be ignored." He demonstrated that there was a statistically significant association between the presence of various lactobacilli and the existence of untreated and therefore possibly active caries. He also showed that among the group of children referred to,[2] twenty-five caries-free children "yielded lactobacillus counts of various magnitudes and 20 per cent of such children had at least one count in excess of 10,000 at some one or other of the annual examinations."

NUTRITIONAL ASPECTS

The dietary pattern between the two groups of Thai children differs to the extent that confectionery is eaten more extensively by the city children and that the rural children eat more fruit and vegetables and unrefined glutinous rice. When setting out to obtain information to enable a pattern of dietary habits to be determined the need of obtaining cooperation from the children was realised. In this matter the class teachers played a most helpful role, aided by the dentist in the area. The data give the broad pattern of the eating habits. Our own observations in the numerous visits to schools and health centres confirmed the recorded data that glutinous rice was eaten extensively in the rural areas. Socio-economic factors may have played a part in the reduction in amount of confectionery consumed by the village children.

Chandrapanond,[8] in a survey of dietary patterns in Thailand, has pointed out that the daily average intake is low in calories (1,800) protein (47 gm.) and calcium (180 mg.) as well as in iron, vitamins B_1, B-complex and C. She has shown that the dietary pattern in some provinces remains severely restricted to whatever foods are available at the time, in contradistinction to the food supply in Bangkok. Basically, provincial diets depend on a large percentage of rice in either white, milled or "glutinous" forms. The average daily diet of the people of Chieng Mai has about 400 calories more than the average for people in Bangkok. Since "glutinous" rice is grown extensively, the farmers have adequate supplies for food. Neilson[9] has observed that the Thais have enough rice to ensure a sufficient caloric intake, but often the glutinous rice is eaten without an accompaniment, when it is made into balls with the fingers and dipped in chili sauce. Because of the method of thresh-

ing, husking and winnowing, it is not completely decorticated and it does not adhere to the teeth.

Table III demonstrates a lower prevalence of caries associated with reduced intake of confectionery, a higher intake of fruits and vegetables and a higher proportion of "glutinous" unrefined rice.

SUMMARY

A group of provincial Thai children were examined clinically and radiographically in order to determine the prevalence of dental caries. Bacteriologic examinations were made of their saliva. A pattern of dietary habits was recorded and indicated a difference between city and rural communities.

The prevalence of dental caries was less in the rural areas and of the whole group was comparable with a special group in Australia, but had a considerably lower prevalence than a group from the general Australian community of the same age. The prevalence of dental caries was lowest in the rural area where less refined carbohydrates and more fruits and vegetables and glutinous rice were eaten.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. Rabieb Rerkasem, Director, and to Dr. Thawon A. Rajathon, Dentist, Nakorn Chieng Mai Hospital, without whose cooperation and assistance this survey could not have been undertaken and to Professor See Sirisinha, Dean, School of Dentistry, Bangkok, Thailand, who undertook the organisation of this journey. Dr. Terb Charudilaka, Associate in Prosthetic Dentistry carried out the task of radiologist with distinction and Tipya Triratana dealt with the various administrative details.

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Letter to the Editor

Sorbitol, an Inhibitor of Intestinal Absorption of Vitamin B_{12}

Dear Sir:

During the last two years several papers were published in *Nature* and *The American Journal of Clinical Nutrition* concerning the augmenting effects of sorbitol on serum vitamin B_{12} levels and on the intestinal absorption of radioactive vitamin B_{12}. Most of these studies were performed in human beings and rats with unphysiologically high doses of vitamin B_{12} and the results were partly unreproducible in other laboratories.

Therefore, we think that it might be of some interest that studies in our laboratories have demonstrated that sorbitol and other carbohydrates are also potent inhibitors of intestinal absorption of vitamin B_{12} and of intrinsic factor in human beings, pigs, rats and guinea pigs if physiologic test doses of radioactive vitamin B_{12} are used.[2–4]

In studies in human beings (^{60}Co-vitamin B_{12} test dose: 0.5 μg.; urinary and faecal excretion test) it was never possible to demonstrate therapeutically significant augmenting effect of sorbitol on absorption of vitamin B_{12}.[2,3] In 30 per cent of the cases administration of 10 gm. L-sorbose or D-sorbitol had an inhibiting effect in normal subjects, partly reducing the intestinal absorption of vitamin B_{12} to the range of pernicious anemia or sprue. This inhibition could be compensated by intrinsic factor in some patients.[3,5] Increasing the dose of sorbose or sorbitol to 30 to 50 gm. is followed in nearly all patients by a reduction of the absorption of vitamin B_{12} which is no longer reversible by intrinsic factor.[3]

In patients with pernicious anemia in remission administration of 10 to 30 gm. of sorbitol interferes with the effect of intrinsic factor on absorption of vitamin B_{12}. For example, the biologic activities of 24 mg. of an Organon intrinsic factor preparation or 5 mg. of a Lederle intrinsic factor concentrate were reduced to 50 per cent after simultaneous oral administration with 0.5 μg. ^{60}Co-vitamin B_{12} and 10 gm. sorbitol.[6,7] The inhibitory action of sorbitol on the intestinal absorption of vitamin B_{12} was compensated by increasing the doses of intrinsic factor to 400 per cent of the values mentioned. However, it was not possible to bring the absorption of vitamin B_{12} back to normal values after sorbitol-inhibition in all patients, especially if higher sorbitol doses (30 to 50 gm.) were used.

Studies in pigs also demonstrated a considerable inhibitory action of 20 gm. L-sorbose on the absorption from an oral test dose of 0.5 μg. ^{60}Co-vitamin B_{12}.[3–5]

A publication from Castle's laboratories on studies in rats clearly shows that administration of an elixir containing 28 per cent sorbitol did not show any improving effect on the absorption of vitamin B_{12} if physiologic test doses of vitamin B_{12} were used in intact or in totally gastrectomized rats.[1]

Recently other studies on the absorption of ^{60}Co-vitamin B_{12} from intestinal loops of the rat have presented evidence that sorbitol in high concentration interfered with the absorption of vitamin B_{12} and that the absorption in rats was still reduced after completion of several months of sorbitol feeding.[9]

In human beings absorption of vitamin B_{12} was still within the range of pernicious anemia sometimes two to three days after a few single oral doses of sorbose or sorbitol.[3,5]

Studies in guinea pigs with a physiologic oral

test dose of 30 ng. ^{60}Co-vitamin B_{12} demonstrate that the inhibitory effect of sorbose on the intestinal absorption of vitamin B_{12} is approximately proportional to the logarithm of the amount of sorbose administered (between zero and 1,500 mg. sorbose).[3,5] Indeed, administration of 100 mg. sorbose proved to have an inhibiting action.

Recent unpublished work on the structural specificity of the inhibitory effect of sorbitol on the intestinal absorption of vitamin B_{92} has shown that other carbohydrates also diminish the absorption of vitamin B_{12} in guinea pigs and rats. Only one, D-fucose, was a much more potent inhibitor than D-sorbitol and none of the tested compounds showed an augmenting effect.[7,8]

The mechanism of action of the inhibitory effect of carbohydrates was studied *in vitro* with a radio-paper-chromatographic technic.[3,5] Sorbitol, sorbose and other carbohydrates, which interfere with absorption of vitamin B_{12}, form a complex with ^{60}Co-vitamin B_{12}, but between 5 and 40 $\times$ 10^6 molecules of the carbohydrate are required for the binding of a single molecule of vitamin B_{12}. Within reasonable concentrations sorbitol does not interfere with the *in vitro* binding reaction between vitamin B_{12} and intrinsic factor.[7] From these observations, and since sorbitol is absorbed and metabolized very rapidly in human beings, it is supposed that the mechanism of action of sorbitol and related compounds is in some way connected with the fixation of the vitamin B_{12}-intrinsic factor complex to the surface of the absorbing intestinal mucosa.

There is no reason to believe that sorbitol could be a substitute for Castle's intrinsic factor and as such augment intestinal absorption of vitamin B_{12}. Repeated administration of high doses of sorbitol in therapy or nutrition, either in human beings or in animals, may produce an inhibition of intestinal absorption of vitamin B_{12} and may ultimately result in the development of a vitamin B_{12} deficiency.

H. C. HEINRICH, DR. MED.
and M. STAAK, DOZENT DR. MED.
Department of Physiological Chemistry
University of Hamburg
Hamburg, Germany

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Reviews of Recent Books

Biochemistry of Nutrition (Biochemie der Ernährung), by K. Lang. Verlag Dr. Dietrich Steinkopff, Darmstadt, 1957, pp. 412, DM 54.

Professor Lang of the Physiochemistry Institute, Mainz University, is to be complimented for the concise presentation of basic biochemical information on carbohydrate, fat, cholesterol, protein, minerals, trace elements and vitamins. He has made ample use of charts (nine) and diagrams (twenty-four) throughout the discussion, and has compiled comprehensive data into 214 tables, which make the book a good source of reference. However, the discussion contains little information on experimental research in nutrition. The book would be useful to the German-speaking physician who wants a general orientation into the biochemistry of nutrition. G. C. CHIU

The Clinical Evaluation of New Drugs, edited by S. O. Waife and A. P. Shapiro. Paul B. Hoeber, Inc., New York, 1959, pp. 223, $7.50.

As a result of the recent tremendous advances in clinical therapeutics there has developed a whole new field of medical research—clinical investigation. This area of endeavor requires specialized knowledge of clinical trials of new drugs and familiarity with certain technics, including design of experiments, suitable controls and clinical pharmacology. The wealth of papers on the evaluation of new drugs is proof enough that the clinical trial has come of age.

Heretofore the literature on the principles and practices of drug testing in man has been scattered in various journals. In this book fourteen distinguished investigators have written short but lucid essays which cover the subject from animal experiments to psychosomatic problems. Included are chapters on the training of investigators, the influence of the scientist on the experiment, ethics, publications, subjective responses and statistical design. About half the book is devoted to concrete problems faced in the field of nutrition and metabolism, cardiology, gastrointestinal diseases, neuropsychiatric, infectious and endocrine disorders, etc.

The chapters are well written and integrated so that the book can be read as a whole. There is no similar book on this important subject and the authors and editors should be congratulated for having so admirably accomplished the task before them—"improving the caliber of the clinical trial in order to define more accurately and rapidly the potential of new drugs." Everyone who studies new compounds in any branch of medicine cannot but profit from this basic, useful and important contribution. J. S.

Books Received

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for a more extensive review.

Clinical Disorders of Hydration and Acid-Base Equilibrium, 2nd edition, by Louis G. Welt, Little, Brown & Co., Boston, 1959, pp. 336, $7.00.

The Heinz Handbook of Nutrition: A Comprehensive Treatise on Nutrition in Health and Disease, edited by Benjamin T. Burton, Blakiston Co., New York, 1959, pp. 439, $5.75.

Gouty Arthritis and Gout. An Ancient Disease with Modern Interest, by Thomas E. Weiss and Albert Segaloff, Charles C Thomas, Springfield, Ill., 1959, pp. 221, $7.50.

A Primer of Water, Electrolyte and Acid-Base Syndromes, by Emanuel Goldberger, Lea & Febiger, Philadelphia, 1959, pp. 322, $6.00.

A Guide to Antibiotic Therapy, by Henry Welch, Medical Encyclopedia, Inc., New York, 1959, pp. 69, $3.00.

Medical Discoveries. Who and When, by J. E. Schmidt, Charles C Thomas, Springfield, Ill., 1959, pp. 555, $14.75.

Antibiotic Therapy for Staphylococcal Diseases, edited by Henry Welch and Maxwell Finland, Medical Encyclopedia, Inc., New York, 1959, pp. 208, $4.50.

Fermente. Hormone. Vitamine. Band I. Fermente, edited by Robert Ammon and Wilhelm Dirscherl, Georg Thieme Verlag, Stuttgart, 1959, pp. 564, DM 81.60 ($19.45).

Lymphocytes and Mast Cells, by Margaret Kelsall and Edward D. Crabb, Williams & Wilkins, Baltimore, 1959, pp. 399, $8.00.

Protein and Amino Acid Nutrition, edited by Anthony A. Albanese, Academic Press, Inc., New York, 1959, pp. 604, $16.00.

Evaluation of Protein Nutrition: A Report of the Food and Nutrition Board, Publication 711 of the National Academy of Sciences—National Research Council, Washington, D. C., 1959, pp. 60, $2.00.

The Megaloblastic Anemias, by Victor Herbert, Grune & Stratton, New York, 1959, pp. 162, $6.00.

Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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JOHN WATERLOW, M.D., *Kingston, Jamaica*

REDUCTION OF SERUM CHOLESTEROL LEVELS BY DIETARY MODIFICATIONS

The isoaloric substitution of unsaturated for saturated fatty acids in the diets of patients with elevated serum cholesterol has been shown to affect a reduction in the levels of this lipid, both in serum and tissues. Experimental and clinical data suggest that the high intake of the unsaturated fatty acids inhibits atherogenesis upon the lowering of serum cholesterol achieved by such diets.

Composition of Plasma Cholesterol Ester in Relation to Coronary-Artery Disease and Dietary Fat. B. Lewis. *Lancet*, 2: 71, 1958.

The proportion of saturated and oleic acids in the fatty acids of cholesterol esters was higher in atheromatous plaques than in plasma. The level in plasma was highest in subjects with established coronary disease and lowest among the South African Bantu who have an exceptionally low incidence of coronary disease.

Feeding experiments using hydrogenated coconut oil and sunflower seed oil showed that the former not only increased the serum content of cholesterol but also lowered the iodine value of fatty acids of cholesterol esters. The reverse was true of feeding with sunflower seed oil. It is believed possible that the primary effect of feeding highly unsaturated fats and therefore increasing the unsaturation of circulating cholesterol esters is to make them more soluble in plasma or to make the cholesterol more susceptible to catabolism to bile acid.

F. E. HYTTEN

Fatty Acid Supplements to the Cholesterol-Fed Rabbit. Effect of Serum Lipids and Atherosclerosis. W. M. Bortz, N. P. Larson and W. H. Civin. *Arch. Path.*, 66: 218, 1958.

Male and female albino rabbits of the Australian strain weighing about $2^1/_4$ to 3 kg. were fed a stock diet containing 2 per cent fat supplemented with 0.5 gm./kg. body weight for five weeks. Subsequently, groups of animals were fed for six more weeks (1) the same diet; (2) supplements of 1 gm. stearic acid per week; (3) 1 ml. oleic acid per week; or (4) 1 ml. linoleic acid per week. The various diets did not influence the lipid phosphorus or the total serum lipoids significantly. Atherosclerotic lesions in the aorta were, however, less marked in the animals receiving unsaturated fatty acids than in those kept on saturated fatty acids.

M. SILBERBERG

Effect of Reduction of Dietary Fat and Additional Ingestion of Corn Oil on Hypercholesterolemia. D. V. Rhoads and M. W. Barker. *Proc. Staff Meet. Mayo Clin.*, 34: 225, 1959.

Nine schizophrenic male subjects with hypercholesterolemia (247 to 331 mg. cholesterol/100 ml.) were the subjects of this study. Following a two-week control period, there was a seventy-day observation period in which the subjects received a diet with a reduced fat content. This was a reduction of from about 100 gm. of animal fat plus 22 gm. of vegetable fat in the routine diet to approximately 57 gm. This was achieved by omitting butter, cream and gravies, substituting skim milk for whole milk, cooking without added fat, etc. During the second period, lasting ninety-two days, the patients received 30 cc. of corn oil with each meal in addition to the reduced fat diet. During the sixty-two–day third period, the routine hospital diet (relatively high in fat) was ingested in addition to 30 cc. of corn oil with each meal. Finally during the fourth period, lasting sixty-six days, they received routine hospital diet alone.

Values for plasma cholesterol decreased in all subjects with the low fat diet alone, decreased further with

the low fat diet plus corn oil, increased but not to control values with the corn oil and routine diet and returned to pretreatment levels when the routine hospital diet was resumed without additional corn oil. Subjects lost weight on the low fat diet alone but regained this when corn oil was added.

The results confirm the observations that moderate reduction of saturated fats in the diet in addition of corn oil produce significant lowering of values for plasma cholesterol in hypercholesterolemia. S. O. WAIFE

The body fat of animals fed unsaturated fatty acids will gradually accumulate a high content of the unsaturated fats. Similar observations have been made in the egg yolks of chickens fed similar dietary sources of these lipids.

Serum-Cholesterol Levels after Consuming Eggs with Increased Content of Unsaturated Lipids. H. Gordon, J. Wilkens and J. F. Brock. *Lancet*, 1:244, 1958.

Feeding trials were conducted on two Bantu men with two types of hen's eggs. In one, "ordinary" eggs were used; the iodine number of the lipids in their yolks averaged 73. In the other, eggs were collected from hens fed on a diet containing sunflower seeds; in these eggs the lipids in their yolks had an average iodine number of 100. There was no effect of the experimental hen diet on production of eggs.

On a basal low fat diet the two men had serum cholesterol levels of about 75 mg. and this rose to 100 mg. per 100 ml. when ten boiled control-egg yolks were added. When these were replaced by "unsaturated" egg yolks the serum cholesterol levels fell slightly to about 95 mg., but there was a marked fall almost to basal levels when ten "unsaturated" eggs fried in sunflower seed oil were added to the diet.

In another experiment without a preliminary control period blood cholesterol rose when the diet contained ten unsaturated egg yolks daily and fell again when the fat content of the diet was reduced. It seems that raising the iodine value of egg yolks from 73 to 100 does not materially affect their cholesterogenic action.

While it is admitted that a greater degree of unsaturation might have been more effective, and more biochemical investigation is required before the full implications of these findings can be assessed, the general conclusion is that the aspect or aspects of dietary fats important in the regulation of serum cholesterol are still unknown. F. E. HYTTEN

Diet and Cholesteremia. I. Development of a Diet for the Study of Nutritional Factors Affecting Cholesteremia in the Rat. N. Nath, R. Wiener, A. E. Harper and C. A. Elvehjem. *J. Nutrition*, 67:289, 1959.

The investigators believed that the rat should prove as useful as any other species for the study of dietary factors affecting the development of atherosclerosis if a diet could be devised that would permit a fairly good rate of growth yet cause a rather rapid rise in concentration of serum cholesterol.

Hydrogenated coconut oil was selected as the source of unsaturated fat. At low levels, increasing quantities of this fat in the diet had little effect on the concentration of serum cholesterol when cholesterol was excluded from the diet. Feeding of cholesterol led to an appreciable elevation in concentration of serum cholesterol and enhancement of symptoms of essential fatty acid deficiency. The addition of corn oil to diets from which cholesterol was excluded resulted in an increase in concentration of serum cholesterol. This is explained by the assumption that the corn oil increased endogenous cholesterol synthesis. However, corn oil fed with cholesterol inhibited the accumulation of cholesterol in both the blood and the liver.

Administration of small amounts of cholic acid along with cholesterol caused a substantial and rapid increase both in the serum and liver cholesterol concentrations. At the same time, good growth was permitted, provided the ration contained less than 0.75 per cent cholic acid. Numerous additional observations are recorded of the effect of varying the amounts of these and other substances in the diet of the rat on serum and liver cholesterol, serum lipid phosphorus, condition of the skin, etc.

The investigators selected a diet containing 25 per cent of hydrogenated coconut oil, 1 per cent of cholesterol and 0.5 per cent of cholic acid as suitable for the study of nutritional factors affecting concentration of serum cholesterol in the rat. FRANK E. RICE

The partially protective effect of a high intake of protein in reducing serum and tissue cholesterol levels may be related to (1) improved liver function with increased biliary excretion of cholesterol metabolites and (2) the higher levels of protein-lipid transport factors in plamsa.

Effects of Dietary Proteins, Methionine and Vitamins on Plasma Lipids and Atherogenesis in Cholesterol-Fed Cockerels. J. Stamler, R. Pick and L. N. Katz. *Circulation Res.*, 6:442, 1958.

Inadequate intake of methionine led to increased hypercholesterolemia and coronary and aortic atherosclerosis in chicks on a cholesterol-oil-supplemented purified ration.

High protein-high vitamin supplementation partially suppressed hypercholesterolemia and atherogenesis in birds on rations of cholesterol oil, of both the purified and commercial mash varieties. High protein supplementation alone tended to produce some of these effects, whereas high vitamin supplementation alone did not.

Chicks fed a purified ration developed less marked hypercholesterolemia and atherogenesis than birds on a commercial feed, even when the former ingested greater quantities of cholesterol. In purified rations containing cholesterol oil, cholesterolemia and atherogenesis tended to be less marked with soy protein than with casein-gelatin (both at the 35 per cent level).

W. H. ABELMANN

Effects of Dietary Protein and Carbohydrate Level on Cholesterolemia and Atherogenesis in Cockerels on a High-Fat, High-Cholesterol Mash. J. Stamler, R. Pick and L. N. Katz. *Circulation Res.*, 6: 447, 1958.

Reduction of protein intake from approximately 20 to 10 to 16 per cent, by addition of sucrose to a high fat, high cholesterol mash, markedly aggravated hypercholesterolemia and atherogenesis in cockerels.

When protein intake was kept at the 20 per cent level by addition of soy protein to the high sucrose, high fat, high cholesterol ration, intensified hypercholesterolemia and atherogenesis did not occur. W. H. ABELMANN

The metabolism, transport and disposal of cholesterol is influenced by the type of fatty acid to which it is esterified. The action of unsaturated fatty acids in reducing serum cholesterol levels is examined in the following reports.

Bile Acid Metabolism, Dietary Fats, and Plasma Cholesterol Levels. S. O. Byers and M. Friedman. *Proc. Soc. Exper. Biol. & Med.*, 98: 523, 1958.

Feeding walnut oil (iodine value 132) to rats reduced the plasma cholesterol and increased the excretion of biliary cholesterol and bile acids. It was wondered whether impaired absorption of cholesterol from the gut might be a factor in causing the hypocholesterolemia seen during the feeding of polyunsaturated fats. Experiments were performed in which the cholesterol content of thoracic duct lymph was measured after feeding fats of differing degrees of saturation. It was found that cholesterol absorption was enhanced by feeding polyunsaturated fats. G. WALKER

Plasma Cholesterol Ester Fatty Acid Composition in Relation to Diet. L. W. Kinsell, G. D. Michaels and G. Fukayama. *Proc. Soc. Exper. Biol. & Med.*, 98: 829, 1958.

Changes in the fatty acid composition of plasma cholesterol esters were observed in five subjects receiving formula diets. Four subjects were given 50 to 100 gm. of ethyl oleate and linoleate at different times and one received a fat free diet. The concentration of mono-enoic acid was highest during the administration of ethyl oleate and with the fat free diet, while the di-enoic acid concentration was greater than that of mono-enoic acid when ethyl linoleate was fed. Tri-, tetra-, penta- and hexa-enoic acid levels were low and varied little with changes in the diet. G. WALKER

Influence of Long Term Fat Feeding on Excretion of Cholesterol-4-C^{14} Metabolites. J. D. Wilson and M. D. Siperstein. *Proc. Soc. Exper. Biol. & Med.*, 99: 113, 1958.

The fecal excretion of the metabolites of injected cholesterol-4-C^{14} was studied in three groups of rats fed diets containing respectively, no fat, 30 per cent corn oil and 30 per cent lard. Animals receiving corn oil showed an increased excretion of non-digitonin precipitable neutral sterol which was largely non-ketonic but was not further identified. Contrary to experience in man the excretion of bile acid was no higher in animals fed corn oil than in those fed lard. G. WALKER

Biological Studies of Dihydrocholesterol. III. Regression of Dihydrocholesterol-Induced Cholecystitis and Cholelithiasis in Rabbits. E. H. Mosbach, M. Berans, R. Kaplan and E. Halpern. *Arch. Path.*, 66: 72, 1958.

Male rabbits weighing 1.5 to 3 kg. were fed a diet of Purina rabbit chow supplemented with 1 per cent dihydrocholesterol and 12 per cent olive oil. As in previous experiments, this diet was fed for periods of from one to nineteen weeks. After that time the rabbits were given the stock ration of rabbit chow only. As long as they were fed the cholesterol diet the rabbits showed cholelithiasis and cholecystitis. Discontinuation of the diet was within fourteen or ninteen weeks followed by resorption of the concretions and a return of the biliary tract to normal conditions. The rate of regression was not influenced by additional administration of 0.25 per cent dihydrocholic acid.

M. SILBERBERG

Biological Studies of Dihydrocholesterol. IV. Effect of Bile Acids and Other Choleretic Agents on Dihydrocholesterol-Induced Cholelithiasis in the Rabbit. E. H. Mosbach, M. Bevans, R. Kaplan, E. Halpern and D. Harris. *Arch. Path.*, 67: 197, 1959.

Chinchilla or albino rabbits were fed a stock diet supplemented with 0.5 per cent dihydrocholesterol, while a group not receiving that supplement remained as control. In addition, both groups of animals received 0.25 per cent of various choleretic agents. Of the latter, deoxycholic and cholic acid completely inhibited cholecystitis and cholelithiasis induced by dihydrocholesterol, while dehydrocholic acid was somewhat less effective. Dreole (p-hydroxyphenyl-salicylamide) gave partial protection, while hyodeoxycholic acid, Zanchol, MA-C_2 and gallogen gave no protection.

M. SILBERBERG

The hypocholesterolemic action of neomycin may be related to partial jejunoilial insufficiency induced by prolonged administration of this drug resulting in absorption deficiencies.

Effect of Neomycin on Serum Cholesterol Level of Man. P. Samuel and A. Steiner. *Proc. Soc. Exper. Biol. & Med.*, 100: 193, 1959.

The oral administration of 1.5 to 2.0 gm. neomycin daily in ten patients was associated with varying initial levels with a fall of serum cholesterol of 17 to 29 per cent (mean 22 per cent) which was evident within two to three weeks after starting the antibiotic and persisted during its continued administration. Lesser effects were obtained with smaller doses. The free:ester cho-

lesterol ratio and the cholesterol:phospholipid ratio were unchanged. The intramuscular injection of amounts of neomycin which could have been absorbed from the oral doses employed had no effect on the concentration of the serum lipids. It was supposed that neomycin acted on the intestinal flora or on gastrointestinal enzyme systems. G. WALKER

Large doses of pyridoxine and nicotinic acid have been demonstrated to reduce serum cholesterol levels. Whether this action is mediated through the enzymatic roles in which these vitamins participate or through some other effect is unknown at present.

Effect in Man of Large Doses of Pyridoxine on Serum Cholesterol. R. B. Failey. *Circulation Res.*, 6:203, 1958.

Sixteen men patients hospitalized for domiciliary care and five women patients with diabetes were given pyridoxine hydrochloride 400 mg./day orally for five to thirty-six days.

Five patients with diabetes treated for seven days showed a significant fall in serum cholesterol from 253 to 234 mg. per 100 ml. ($p < 0.05$). Ten subjects without diabetes treated comparably failed to show a significant fall. Five of six subjects without diabetes treated for a maximum of thirty-six days showed a fall in serum cholesterol; the mean value of the group decreased significantly from 257 to 236 mg., and rose again to 242 mg. per 100 ml. during a follow-up control period.

W. H. ABELMANN

Effect of Nicotinic Acid on Serum-Lipids in Normal and Atherosclerotic Subjects. P. A. Galbraith, W. F. Perry and R. E. Beamish. *Lancet*, 1:222, 1959.

Eight male medical students of average age twenty-two years, and fourteen subjects with atherosclerosis aged thirty-six to seventy-one years were given nicotinic acid 1 gm. three times daily while remaining on their normal diets. In the normal subjects the mean serum cholesterol fell from about 190 mg. to 160 mg. per 100 ml., and from about 340 mg. to 260 mg. per 100 ml. in the patients with atherosclerosis. It rose again as soon as administration of nicotinic acid was stopped. Total lipids and β-lipoprotein levels also fell under the influence of nicotinic acid. The mechanism is unknown. F. E. HYTTEN

Influence of Cobaltous Chloride on Aortic Atheromatosis and Plasma Lipid Pattern in Cholesterol-Fed Chickens. D. M. Tennent, C. W. Muschett, G. W. Kuron, W. H. Ott and H. Siegel. *Proc. Soc. Exper. Biol. & Med.*, 98:474, 1958.

White Leghorn cockerels eight weeks of age were fed a diet containing 2 per cent cholesterol and 5 per cent cottonseed oil for eight weeks. In order to test the effect of cobalt, cobaltous chloride in amounts from 0.001 per cent to 0.25 per cent was added to the diet or injected subcutaneously. The aortas were studied for the presence of atheromatous lesions. In the plasma total cholesterol and lipid phosphorus were determined. Moreover, the pancreas and other endocrine organs were examined. Feeding of cobalt reduced the incidence and attenuated the severity of the aortic lesions and lowered the levels of blood cholesterol and of beta-lipoproteins. The effect of cobalt on concentrations of lipid phosphorus was inconsistent. Cobalt feeding had no effect on the thyroid or on the structure of the alpha cells of the pancreas. Subcutaneous administration of cobalt aggravated the development of atheromatosis and increased the concentration of cholesterol in the blood. It therefore appears that the effect of dietary cobalt is due to its action in the intestinal canal.

M. SILBERBERG

Inhibition of Dietary Hypercholesteremia and Atherogenesis in the Chicken by a New Synthetic Compound. T. R. Blohm, L. J. Lerner, T. Kariya and M. E. Winje. *Circulation Res.*, 6:260, 1958.

A synthetic compound, 1,1-bis-(p-methoxyphenyl)-2-(p-guanylphenyl)-ethylene hydrochloride (MER-13), given in the diet at a level of 0.1 per cent, inhibited the development of hypercholesteremia and aortic and coronary atherosclerosis in cockerels fed cholesterol. Under these conditions it had no effect on food consumption, body weight, size of comb or weight of testes. MER-13, like β-sitosterol, did not increase the rate of regression of established hypercholestermia in chickens when cholesterol was removed from the diet. It is probable that it inhibits intestinal absorption of cholesterol. Because of its water solubility and potency, MER-13 or similar materials may possess therapeutic advantages over steroid inhibitors of cholesterol absorption. W. H. ABELMANN

BIOCHEMICAL ASPECTS OF CHOLESTEROL AND ATHEROGENESIS

The deposition of cholesterol in the arterial wall probably occurs in areas of increased formation of mucopolysaccharides. The inhibition of production of the latter substances by fibroblasts has been shown to result during administration of cortisone. The reduced concentration of mucopolysaccharide in cortisone-treated animals may be a factor in the inhibition of formation of atheroma.

The Effects of Cortisone upon Aortic Intimal Repair in the Hypercholesteremic Rabbit. J. T. Prior, R. F. Rohner, F. A. Camp and H. Rustad. *Arch. Path.*, 67:159, 1959.

White rabbits of the New Zealand strain, eight to ten weeks old and weighing about 2,200 gm. were fed a diet of Purina rabbit chow supplemented with 1 gm. of cholesterol per day for periods ranging from one day to twelve weeks. Animals fed the regular diet served as controls. In the rabbits of one group the abdominal aorta was cautiously traumatized with an inserted 24-gauge needle

which was moved circularly inside the vessel. Before or after the injury the animals received intramuscular injections of cortisone acetate ranging in dosages from 0.1 mg. to 15 mg. per rabbit daily. At necropsy the injured tissue was removed as a whole and the tissue changes were studied microscopically. The blood cholesterol values were markedly elevated but it was felt that the administration of cortisone was not particularly responsible for unusually high elevations. Gross and microscopic examination showed that cortisone prevented the formation of aortic plaques in those rabbits with hypercholesteremia which survived two months. The inflammatory and, in the traumatized animals, the reparative reactions also were delayed, and a lack of lipophages in these areas typical of animals with hypercholestermia not treated with cortisone was striking. The latter finding is thought to explain the retardation of the atherogenic process observed.

M. SILBERBERG

Uptake of Radioactive Sulphur by Various Tissues of Normal and Cholesterol-Fed Rabbits. R. C. Buck and F. C. Heagy. *Canad. J. Biochem. & Physiol.*, 36: 63, 1958.

Male rabbits weighing 2 to 3 kg. were fed a stock diet of Master Feeds ad libitum; one group received the same ration supplemented by 1 per cent cholesterol for a period of four months. Two days before being sacrificed the animals were given one injection of 650 μc./kg. body weight of neutral radioactive sodium sulfate (S^{35}). At autopsy atheromatous plaques of varying size were seen. The concentration and activity of S^{35} in the aortas, intestine, kidney, stomach, salivary gland, liver and costal cartilage were determined. The specific activity and concentration of sulfur in the aortas of cholesterol-fed rabbits was significantly higher than in those fed the stock diet, the specific activity being twice as high and sulfur concentration about 25 per cent higher in the cholesterol-fed rabbits. Since the incorporation of S^{35} parallels the intensity of staining for acid polymucosaccharides, chondroitin sulfate seems to be primarily involved in taking up S^{35}.

M. SILBERBERG

Species Differences in Cholesterol Biosynthesis by Arterial Tissue. D. L. Azarnoff. *Proc. Soc. Exber. Biol. & Med.*, 98: 680, 1958.

Aortas and coronary arteries of human beings and aortas of various species were studied in regard to their ability to incorporate C^{14}-labeled acetate into cholesterol. Slices of arteries were placed in a phosphate buffer containing acetate-1-C^{14} and incubated for two hours. Subsequently, cholesterol was added, extracts were made and the incorporation of C^{14} into cholesterol was studied *in vitro*. While arteries of guinea pigs, rabbits and chickens were capable of synthesizing C^{14} and cholesterol, those of human beings, dogs, cats and rats failed to do so. These species differences seem to indicate that not only the lipids of the plasma, but also the metabolic activity of the tissue itself play a major role in the formation of atheromatous plaques.

M. SILBERBERG

Response of Arterial Wall to Intramural Cholesterol. M. G. Notsky and T. B. Clarkson. *Proc. Soc. Exper. Biol. & Med.*, 98: 773, 1958.

In white Leghorn cockerels weighing 1 to 1.5 kg., the brachiocephalic artery was exposed, and 0.2 ml. of a 20 per cent suspension of crystalline cholesterol in 0.9 per cent saline was injected into the wall of the vessel. The animals thus treated were placed on one of the following rations: (1) stock diet of mash; (2) stock diet enriched with 8 per cent lard; (3) stock diet supplemented with 8 per cent corn oil; and (4) stock diet enriched with 4 per cent lard, 4 per cent corn oil and 1 per cent cholesterol. The birds were sacrificed at intervals varying from two to ten weeks, and the brachiocephalic arteries were studied microscopically. At the site of the injection, the adventitia and media showed granulomatous nodules composed of proliferating collagenous tissue with foreign body giant cells and cholesterol clefts. Such granulomas were, however, most striking in the intima; they partly obliterated the vascular lumen. The development and structure of these plaques were not affected by the various diets. In control animals in whom saline had been injected no granulomas formed.

This reviewer feels that the lesions produced constitute lipogranulomas rather than arteriosclerotic plaques in spite of a certain morphologic similarity between the two.

M. SILBERBERG

Absorption of cholesterol occurs principally in the esterified form. The dynamics of this process require further study as efforts continue in the direction of inhibiting absorption or increasing the rate of degradation of this lipid.

Mechanism of Cholesterol Absorption. II. Changes in Free and Esterified Cholesterol Pools of Mucosa after Feeding Cholesterol-4-C^{14}. L. Swell, E. C. Trout, Jr., J. R. Hopper, H. Field, Jr. and C. R. Treadwell. *J. Biol. Chem.*, 233: 49, 1958.

Rats with fistulas of the thoracic duct were given test meals containing 40 to 44 mg. of cholesterol-4-C^{14} and lymph collected over periods of six, twenty-four or forty-eight hours. After forty-eight hours, 10.8 mg. or 27.3 per cent of the administered C^{14}-cholesterol was found in the lymph in a total lymphatic excretion of 32.3 mg. of cholesterol. Total cholesterol in the wall of the intestine was about 20 mg. of which about 6 per cent was normally esterified. During the height of absorption of the labeled cholesterol, six hours after the test meal, up to 10 per cent of the intestinal cholesterol was esterified. The level of cholesterol-C^{14} in the intestine decreased 2.3 mg. between twenty-four and forty-eight hours after administration, and a corre-

sponding increase in labeled cholesterol was found in the lymph. Specific C^{14} activities of free cholesterol were higher in lymph than in any of the intestinal segments studied, but were lower than the esterified fractions of either lymph or any part of the intestine, while the specific activity of esterified cholesterol in lymph was about equal to that found in the middle or most active portion of the small intestine. These experiments show that free cholesterol absorbed into the intestine is diluted with a large pool of endogenous free cholesterol, most of which is esterified before entering the lymph, while esterified cholesterol is absorbed without significant dilution. A metabolic pool of free cholesterol exists in the intestinal mucosa. M. K. HORWITT

Absorption of Cholesterol Esters in the Lymph-Fistula Rat. G. V. Vahouny and C. R. Treadwell. *Am. J. Physiol.*, 195: 516, 1958.

Free and esterified cholesterol fractions in lymph of the thoracic duct of rats were determined after the administration of emulsions containing saline, albumin, taurocholate, oleic acid and free cholesterol, or fatty acid esters of cholesterol. Total cholesterol in the lymph increased within three hours after feeding free cholesterol or the natural esters of cholesterol. Cholesterol butyrate was absorbed as well as free cholesterol, while the esters of oleic and linoleic acids were absorbed slower and less efficiently. Saturated acid esters, laurate and stearate were absorbed slowly and inefficiently. Administration of cholesterol trimethylacetate, which is resistant to the hydrolytic action of pancreatic cholesterol esterase, did not result in any increase in cholesterol in the lymph above the control level. Throughout all groups in which absorption of cholesterol occurred, the absorbed cholesterol in lymph was esterified from 87 per cent to 95 per cent. The data are in agreement with the concept that dietary esters of cholesterol are hydrolyzed in the intestinal lumen prior to absorption of the cholesterol moiety as free cholesterol.

AUTHORS

Atherosclerosis in Rhesus Monkeys. I. Hypercholesterolemia Induced by Dietary Fat and Cholesterol. G. E. Cox, C. B. Taylor, L. G. Cox and M. A. Counts. *Arch. Path.*, 66: 32, 1958.

Thirty male and female Rhesus monkeys weighing about 8 lb. each received diets high in fat with supplements of cholesterol for eight to ninety-eight weeks. The experimental diet contained 10 gm. or 14 gm. fat (butterfat or vegetable oil) and supplements of 1.5 gm. cholesterol with or without an additional egg. The ration was fed ad libitum once a day. A stock diet containing 2 or 3 gm. fat and no cholesterol, or only a trace, served as a control ration. In the test animals blood cholesterol rose to 300 to 600 mg./100 ml. within three months. There were considerable variations, some monkeys being more susceptible and others less, to the harmful effects of the high fat, cholesterol-enriched diet. Serum fat levels on the other hand were normal or subnormal even in the presence of hypercholesterolemia. Dietary fat alone did not produce hypercholesterolemia even after consumption of the high fat ration for several months. The aortas of the monkeys with hypercholesterolemia showed microscopic lipid deposits. These changes are thus thought to be specifically due to ingestion of cholesterol and not of fat. M. SILBERBERG

Several studies have suggested that serum cholesterol levels are in part, genetically determined. Observations on the dietary fat and cholesterol intake within the families involved in some surveys indicate that elevated levels of this lipid may occur despite normal or low intakes.

Heredity, Environment, and Serum Cholesterol. A Study of 201 Healthy Families. L. E. Schaefer, D. Adlersberg and A. G. Steinberg. *Circulation*, 17: 537, 1958.

Serum cholesterol levels (Sperry and Schoenheimer) were analyzed in a sample of healthy city employees and their families, totalling 1,236 persons, including 775 members of 201 families.

The average serum cholesterol level for males was constant for the ages two through ninteen years, increased markedly thereafter to age thirty-two and then remained essentially constant to age sixty. The average serum cholesterol level for females remained constant through age thirty-two years and then increased to age fifty-eight.

Only eight of 337 children of 182 families with normal parents had hypercholesteremia. Of the thirty-six children of ninteen families having one parent with hypercholesteremia, six had hypercholesteremia. These findings are considered consistent with the hypothesis that hypercholesteremia is due to a dominant gene with incomplete penetrance.

The serum cholesterol levels of parents showed no correlation, while those of parent and child and those of siblings showed significant positive correlation. These findings support the hypothesis that there are genetic determinants of serum cholesterol levels.

W. H. ABELMANN

Variations in the Serum Total Cholesterol and Hematocrit Values in Normal Women During the Menstrual Cycle. *Scandinav. J. Clin. & Lab. Invest.*, 11: 1, 1959.

Twenty-nine women ranging in age between twenty and forty-one years, but the majority between twenty and twenty-five years, were followed during their menstrual cycles. There was a postovulatory fall in serum cholesterol values which was statistically significant. Highest values were noted with ovulation. The authors verified a premenstrual rise and a menstrual fall which had been previously reported. These variations harmonize with other physiologic phenomena occurring during the menstrual cycle. Similarly, a premenstrual fall in hematocrit value was statistically significant.

S. O. WAIFE

The deposition of cholesterol within the intimal lining of a fabric tube graft in the aorta is an unusual observation. Cholesterol is believed to be transported into the intima within the beta-lipoproteins of the plasma. At high plasma levels the rate of transport into the intima is increased with more cholesterol remaining in the tissue.

Lipid Changes Produced by Chronic Hypercholesterolemia in Nylon and Orlon Replacements of Canine Thoracic Aorta. G. L. Jordan, Jr., M. E. de Bakey and B. Halpert. *Proc. Soc. Exper. Biol. & Med.*, 99: 484, 1958.

A segment of the aorta was removed from six mongrel dogs. The defect was closed by a nylon or orlon fabric tube using running sutures of silk for the end-to-end anastomoses. After two weeks, 1 mc./kg. body weight of radioiodine was administered parenterally and the dogs were fed a diet of 70 per cent horse meat, 25 per cent Purina chow and 5 per cent cholesterol. Serum cholesterol was determined weekly for periods up to sixty-two weeks. The cholesterol concentrations ranged from 358 to 880 mg. per 100 ml. Two dogs with the highest serum cholesterol concentrations developed yellow plaques containing foam cells between delicate fibers in the intima which had relined the grafts. No changes were noted in the intima of the remainder of the aorta. In homografts of aortic tissue on the other hand such plaques could be produced more readily and more frequently than in the fabric grafts. This difference may be perhaps due to difference in structure and wettability of these different types of tissue surfaces. Although morphologically the organized lipoid plaques produced in the intima of the fabric graft have certain features in common with those seen in atherosclerosis, and although both may arise from organizing blood clots, it seems doubtful, if one should consider the lesions as atherosclerotic in the strict sense.

M. Silberberg

The Esterification of Cholesterol with Palmitic Acid by Rat Liver Homogenate. S. Mukherjee, G. Kunitake and R. B. Alfin-Slater. *J. Biol. Chem.*, 230: 91, 1958.

Cholesterol esterases apparently occur in a number of tissues. This report verifies the presence of an enzyme in the liver of the rat which can facilitate the synthesis of a cholesterol ester of palmitic acid. This enzyme requires the presence of adenosine triphosphate (ATP) and coenzyme A (CoA). Increases in the amount of incorporation of radioactivity in the cholesterol ester fraction in the incubated system were obtained when (1) CoA and ATP were present with C^{14}-labeled cholesterol and sodium palmitate, (2) when C^{14}-labeled palmityl-S-CoA and cholesterol were present and ATP and CoA were omitted, and (3) when labeled palmityl-S-CoA and labeled sodium palmitate were included in the presence of ATP and CoA. No significant esterification was observed in the absence of the co-factors CoA and ATP. These results support the theory that this enzymatic esterification is the result of two distinct steps, the activation of the fatty acid by ATP to form the acyl-CoA derivative which then combines with the sterol to form the ester. M. K. Horwitt

Effect of the Energy to Protein Ratio on Serum and Carcass Cholesterol Levels in Chicks. M. Kokatnur, N. T. Rand and F. A. Kummerow. *Circulation Res.*, 6: 424, 1958.

One day old chicks were fed a balanced diet containing either corn oil ("soft" fat) or beef tallow ("hard" fat) as source of fat and variable amounts of protein and glucose. Thus the ratio of total metabolizable energy to protein varied from 8 to 23. After three to four weeks serum and carcass cholesterol and total carcass fat were measured.

Low energy to protein ratios depressed serum cholesterol and total carcass fat irrespective of the type of fat or protein in the diet. "Soft" fats were found to depress significantly serum cholesterol levels in diets of high energy to protein ratio but not in diets of low energy to protein ratio. W. H. Abelmann

Ultraviolet Spectrophotometric Method for the Determination of Cholesterol. B. I. Weigensberg and G. C. McMillan. *Am. J. Clin. Path.* 31: 16, 1959.

A spectrophotometric method to determine free and total cholesterol after precipitation of the cholesterol as its digitonide is described. Now that there are improved spectrophotometers that permit measurements of absorption in the 200 to 210 $\mu\mu$ region without the use of vacuum ultraviolet methods, measurement of the absorption band of cholesterol in the ultraviolet region makes the method three times more sensitive than the Schoenheimer-Sperry method, as used routinely. Another advantage is the fact that the sulfuric acid-acetic anhydride reagent of the Liebermann-Burchard reaction is not required. The method is reported not susceptible to time, light, moisture, reagent concentration and other variables. A striking feature of this method is that the cholesterol does not react with any reagents other than digitonin. According to the authors, their procedure is adaptable for determining the free and total cholesterol extracted from serum, plasma or tissues, such as atherosclerotic or normal blood vessels, which only contain trace amounts of digitonin-precipitable sterol hormones or vitamins.

E. Cohen

Absorption of H^3-β-Sitosterol in the Lymph Fistula Rat. L. Swell, E. C. Trout, Jr., H. Field, Jr. and C. R. Treadwell. *Proc. Soc. Exper. Biol., & Med.*, 100: 140, 1959.

Rats were fed an emulsion containing oleic acid, sodium taurocholate, albumin, glucose and 44 mg. H^3-β-sitosterol. Five per cent of the sitosterol was recovered from the wall of the intestine six hours after administration but only traces were present after forty-eight hours. Virtually all the sterol was in the unesterified

form. Although only traces of radioactivity were detected in the lymph of the thoracic duct at any time only 60 per cent of the sitosterol was recovered from the wall and contents of the intestine after forty-eight hours, suggesting that an appreciable amount had been degraded to a non-digitonin precipitable form.

G. WALKER

DIETARY FACTORS AND EXPERIMENTAL CANCER

Modification of the rate of spread of experimentally induced cancer in animals by dietary factors, including specific amino acids and antimetabolites, is a potentially rewarding field of investigation. The response of cancer to indole, tryptophan and high protein diet administered to rats has been examined.

Effect of a High Protein and High Nucleic Acid Diet on Occurrence of 2-Acetylaminofluorine-Induced Cancer in Rats. W. F. Dunning and M. R. Curtis. *Proc. Soc. Exper. Biol. & Med.*, 99:88, 1958.

Female rats of the Fischer 344 line about five months of age were fed isocaloric portions of a synthetic diet containing a base of 45 per cent casein in which the latter was substituted by 1, 2 and 5 per cent desoxyribonucelic acid and 5 per cent yeast, respectively. These diets were supplemented by 0.62 per cent 2-acetylaminofluorine. After periods of from ten to fourteen months and depending on the diet hepatomas developed in 67 to 92 per cent of the rats, mammary in 4 to 25 per cent and cancer of the ear in 33 to 75 per cent. The substitution of high nucleic acid yeast for casein significantly increased the survival of the rats but it did not prevent the induction of neoplasms. Substitution of desoxyribonucleic acid or of yeast for casein had no effect on the survival nor on the incidence of tumors induced by the carcinogen. The high protein diet, however, accelerated the appearance of squamous cell carcinoma of the external auditory canal in rats fed acetylaminofluorine.

M. SILBERBERG

The Role of Indole in Incidence of 2-Acetylaminofluorene-Induced Bladder Cancer in Rats. W. F. Dunning and M. R. Curtis. *Proc. Soc. Exper. Biol. & Med.*, 99:91, 1958.

Female rats of the Fischer 344 strain four to five months old received a daily portion of 7 gm. of a synthetic diet containing about 26 per cent protein, 52 per cent carbohydrate and 15 per cent fat plus adequate amounts of minerals and vitamins. These diets were supplemented by 0.06 per cent 2-acetylaminofluorine. The variants in the diets were the amounts of DL-tryptophan, indole and indole acetic acid. After eleven to fourteen months hepatomas, tumors of the urinary bladder and mammary gland, and cancers of the ear developed in many rats. Cancers of the liver were found in 83 to 91 per cent of the rats fed 1.4 per cent DL-tryptophan, 1 per cent indole acetic acid, 0.8 per cent indole or 1.6 per cent indole. Those fed indole showed cholangiomas or adenocarcinomas, the remaining were the liver-cell type of cancers. Papillary or squamous cell carcinomas of the urinary bladder were found in 83 per cent of the rats fed indole, in 75 per cent of those fed DL-tryptophan and in 50 per cent of those fed indole acetic acid. Indole thus appeared as effective as tryptophan in the initiation of urinary bladder tumors induced by acetylaminofluorine.

M. SILBERBERG

The inhibition of ethionine-induced cancer by methionine is the converse of tumor-inhibiting action of the antimetabolites. The protection afforded by methionine is due to the prevention from the hepatotoxic action of its congener, ethionine.

The Prevention of Ethionine-Induced Carcinoma of the Liver in Rats by Methionine. E. Farber and H. Ichinose. *Cancer Res.*, 18:1209, 1958.

Male and female white rats of the Wistar strain weighing 150 to 260 gm. were fed a basal diet containing 16 per cent casein, plus sucrose, corn oil, salt mixture, vitamins and 0.25 per cent DL-ethionine. Group 1 received this stock diet with supplements of 0.3 per cent DL-methionine and 0.3 per cent choline chloride; rats of group 2 were fed the stock diet with supplements of 0.2, 0.6 or 0.8 per cent methionine; group 3 received the stock diet with addition of 0.6 or 0.8 per cent methionine, 0.6 or 0.8 per cent choline chloride with 0.8 per cent betaine chloride. These diets were fed for eight months, after which period the stock diet was given for another two months. The rats were then sacrificed and the changes in the liver were studied grossly and microscopically with special reference to the development of cancers. Supplements of 0.6 or 0.8 per cent methionine completely prevented the changes in the liver and cancers induced by ethionine, while lower doses of methionine as well as of choline were only partially effective in protecting the rats against damage to the liver. Apparently the carcinogenic effect of ethionine is due to interference with the methionine metabolism.

M. SILBERBERG

Dietary protein deficiencies in combination with chemical irritants are capable of producing damage to the liver which leads to formation of hepatoma. The reduction of liver damage and resultant hepatic malignancy by the administration of copper salts is an observation requiring further study.

The Effect of Copper Acetate on d-Dimethylaminoazobenzene Carcinogenesis in the Rat. J. S. Howell. *Brit. J. Cancer*, 12:594, 1958.

Male and female rats of a heterozygous strain, two and six months old at the beginning of the experiment, were divided into several experimental groups. All rats received the Thompson diet which is very rich in nitrogen-free extract, that is, mainly in starch and which also has a relatively high fat content. It does

not contain more than 10 per cent protein, principally zein which is of poor biologic value and deficient in lysine and tryptophan. Dry crystalline d-dimethylaminoazobenzene (DMAB) was added to this diet in a concentration of 0.09 per cent. Some groups of animals received supplements of 0.5 per cent powdered copper acetate and/or 2 per cent ferric citrate. The feeding experiments varied in the supplements. Copper acetate significantly retarded development of tumors in the liver caused by DMAB. As determined by biopsy of the liver in rats in whom hepatoma did not develop, damage to the liver and specifically cirrhosis was also less frequent after ingestion of copper.

M. SILBERBERG

Studies on the Inhibition of Growth of an Implanted Fibrosarcoma in Rats. The Effect of Fat in the Diet with and Without Injections of Guinea Pig Serum. E. Jameson, R. M. Ryan and P. I. Kramer. *J. Nutrition*, 67:469, 1959.

Randomly bred and inbred AXC9935 Irish gray rats, 75 to 125 days old were intraperitoneally injected with guinea pig serum or Ringer's Solution while on fat-enriched (cotton seed oil or butter) diets. The rats bore subcutaneous trochar transplants of ACMCA, fibrosarcoma. Significance of the treatments was determined on the fortieth day after tumor implantation. Control rats on a fat diet alone had a significant (at the 50 per cent level) reduction of the number of tumor "takes" or growth of tumor. Injections of guinea pig serum enhanced the effect of fat in the diet. Statistically, a dietary supplement of methyl linoleate alone had as great an effect as 15 per cent cottonseed oil diet; 4 per cent butter supplement was not inhibitory. Injections of guinea pig serum, alone, significantly inhibited trochar implanted fibrosarcoma growth in rats. However, 30 per cent of the tumors developed if injections were stopped. Fat in the diet increased the activity of guinea pig serum. It was concluded that essential fatty acids are required for maximal host resistance to the $ACMCA_2$ fibrosarcoma. The mechanism of the inhibition is not explained by the experiments described. Unpublished work of the authors suggested to them that gamma-1-globulin is protected during x-radiation by fat-enriched diets. Just how that affects the immune mechanism is not discussed. Nevertheless, nutritionist or immunologist may find the authors' experiments as provocative as may the cancer researcher.

E. COHEN

The Modifying Influence of Diet and the Physical Environment on Spontaneous Tumour Frequency in Rats. C. Gilbert, J. Gillman, P. Loustalot and W. Lutz. *Brit. J. Cancer*, 12:565, 1958.

Male and female albino rats of the GG, Copenhagen and Utrecht strains were studied with reference to the effects of diets on the incidence of spontaneous tumors. The basal diet I contained the following main ingredients: 60.4 per cent carbohydrate, 15.2 per cent protein and 11.3 per cent fat. The experimental high carbohydrate diets II and III contained 78 per cent or 65.9 per cent carbohydrate respectively, 12.3 per cent or 14.8 per cent protein and 8.1 per cent or 8.6 per cent fat, while the high protein diet IV contained 3.6 per cent carbohydrate, 81.6 per cent protein and 10.2 per cent fat. Of all neoplasms observed 80 per cent were found in the endocrine glands. Pheochromocytomas of the adrenal gland were the most common growths in both sexes, with interstitial tumors of the testes in males and fibroadenomas of the breast in females next. Neoplasms also were observed in pancreas, ovary, thyroid, uterus, lung and other viscera. In seventy-four per cent of the males and 50 per cent of the females tumors eventually developed. The high protein and high carbohydrate diets delayed the onset and decreased the over-all incidence of tumors, particularly of pheochromocytomas, while the high carbohydrate diet increased the rate of tumor formation in the pituitaries of males and of uterine cancers.

M. SILBERBERG